IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	Petkovich *et al.*
Serial No.:	09/668,482
Filing Date:	September 25, 2000
Title:	Retinoid Metabolizing Protein
Art Unit:	1652
Examiner:	Elizabeth Slobodyansky, Ph.D.
Atty's Docket No.:	32391-2005

OFFICIAL

The Commissioner of Patents and Trademarks
Washington, D.C. 20331
U.S.A.

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this correspondence, totaling 70 pages, is being facsimile transmitted at **703.872.9306** to the USPTO on the date set forth below:

John C. Hunt, Registration No. 36,424 — October 15, 2003 (Date)

CONFIDENTIALITY NOTE:

This message is CONFIDENTIAL and is legally privileged. It is intended only for the person(s) or organization(s) named above and any other use or disclosure is strictly forbidden. If this message is received by anyone else, please notify us at once by telephone (collect, 416.865.8121) and return the original by mail to:

John Hunt
Torys LLP (Customer No. 33721)
Suite 3000, 79 Wellington Street
West
Box 270, TD Centre
Toronto, Ontario Canada
M5K 1N2

Thank you

PETITION UNDER 37 CFR §§ 1.181 & 1.182

Dear Sir:

This is a petition made in a situation not specifically provided for in the regulations. The undersigned submitted a response to an action on August 11, 2003, and the PTO failed to deduct required fees from Applicant's Attorney's Deposit account, as authorized in the response. In view of th detail d

statement of facts set out below, the undersigned hereby requests the PTO to deduct the required fees from the Deposit account and to accept the response as timely received.

Statement of Facts

Timeline of events:

1. A non-final action was issued by the PTO on February 13, 2003, copy included as Exhibit A.
2. The undersigned submitted a response to the action to the PTO by facsimile on August 11, 2003. A copy of the response, which included a petition for extension of time, is included as Exhibit B.
3. On August 19, 2003, the PTO mailed a notification of "Informality re payment of fee" to the undersigned at the address of his former firm, Blake, Cassels & Graydon LLP. A copy is included as Exhibit C.
4. On September 3, 2003, the undersigned submitted a Power of Attorney in his favor. Included as Exhibit D is the complete submission with a copy of the receipt card date stamped by the PTO.
5. On October 14, 2003 the undersigned's former firm faxed a copy of the PTO notification of August 19, 2003 to the undersigned at his current address. A copy of the fax is included as Exhibit E.

Points to be reviewed and action requested:

6. The PTO notification of August 19, 2003 indicates that "the amendment is considered incomplete in that the funds in Deposit Account No. are insufficient to cover the entire fee due. A period of one month was granted for responding to the notification, which period ended on September 19, 2003.
7. Included as Appendix F is a copy of the Deposit Account Statement for Deposit Account No. 502,651, as obtain d from the PTO Web site on October 14, 2003. The Statement indicates that for the entire month of

August, the balance in the account did not go below $1,655.00.

8. As indicated in Applicant's Petition for extension of time, included as part of Exhibit B, the box which states "the Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 502651" is checked.

9. Applicant thus submits that the PTO was properly authorized to charge all required fees to Deposit Account Number 502651 and that the account included sufficient fees.

10. Applicant hereby requests that: the extension fee of $930.00 be deducted from Deposit Account Number 502651; the IDS fee of $180.00 be deducted from Deposit Account Number 502651; that the PTO notification be withdrawn as though it had never been issued; and Applicant's response of August 11, 2003 be entered and returned to the Examiner for further prosecution. Applicant further requests that, if a notice of abandonment has been issued by the PTO, that such notice also be withdrawn.

11. The Director is hereby authorized to charge any other fee which may be required for this submission, including any petition fee, or credit any overpayment, to Deposit Account Number 502651.

Should any Patent Office Official want to telephone, the call should be made to John C. Hunt (Registration No. 36,424) at (416) 865-8281.

Yours very truly,

John C. Hunt
Registration No. 36,424

October 15, 2003
Date

Torys LLP (Customer No. 33721)
Suite 3000 - 79 Wellington Street West
Box 270, TD Centre
Toronto, Ontario
M5L 1A9
Canada

Exhibit A

Page 1 of 25 pages

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/668,482	09/25/2000	P. Martin Petkovich	57600/00035	3039

7590 02/13/2003

John C Hunt Blake Cassels & Graydon
Intellectual Property Group LLP
Commerce Court West
P O Box 25
Toronto, ON M5L 1A9
CANADA

EXAMINER
SLOBODYANSKY, ELIZABETH

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 02/13/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)
	09/668,482	PETKOVICH ET AL.
	Examiner	Art Unit
	Elizabeth Slobodyansky	1652

-- *The MAILING DATE of this communication appears on the cover sheet with the correspondence address* --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *83-95,97-102 and 104-112* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *83-95,97-102,104-112* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *13* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

Art Unit: 1652

DETAILED ACTION

The finality of the Office action mailed August 13, 2002 is hereby withdrawn in view of the new ground(s) of rejection.

Claims 83-95, 97-102 and 104-112 are pending.

During a telephone conversation conducted on February 12, 2003, Dr. John Hunt requested an extension of time for three MONTH(S) and authorized the Commissioner to charge Deposit Account No. 02-2553 the required fee of $ 920.00 for this extension.

Specification

The disclosure is objected to because of the paper and computer readable forms of the Sequence Listing are not identical. The paper copy contains 35 sequences while the computer readable form has 43 sequences.

A substitute paper copy of the Sequence Listing identical to the computer readable form in the file is required. It should be accompanied by the statement the two forms are identical and by the amendment directing the entry of a substitute Sequence Listing.

Claim Objections

Claims 83, 89 and 90 are objected because of the following.

In claims 83 and 90, the proper recitation of the Markush group requires "or" before the last member of the group (line 8).

Claim 89 is objected because of the following. The Markush group requires "or" before "SEQ ID NO:32" at two occurrences.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 97-102 and 104-112 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims encompass all polypeptides of any function or that oxidize/hydroxylate retinoid and that bind to any antibody specific for SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:32. The Examiner is unable to locate adequate support in the specification for such polypeptides. Thus there is no indication that polypeptides encompassed by claims 97-102 and 104-112 were within the scope of the invention as conceived by Applicants at the time the application was filed.

Accordingly, Applicants are required to cancel the new matter in the response to this Office Action.

Claims 83, 85-90, 92-95, 97-102 and 104-112 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims recite "a conservatively substituted amino acid variant" of the amino acid sequences encoded by SEQ ID NOs: 3, 5 or 31 or encoded by a DNA that hybridizes thereto or to a degenerate variant thereof under specific conditions. This amounts to any structure having the same function as a protein encoded by SEQ ID NOs: 3, 5 or 31. The structural limitations are insufficient because while a substitution is required to be conservative any amino acid residue in the sequence and any number thereof can be substituted resulting in a completely novel structure that is not described. This is equivalent to a claim with no structural limitations wherein an enzyme is defined by the function only. Furthermore, the function described only as "oxidizes/hydroxylates a retinoid" encompasses many different activities and substrate specificities. In addition, a genus of degenerate variants of SEQ ID NOs: 3, 5 or 31 is enormous.

Claims 83, 85-90, 92-95 and 107-112 are directed to polypeptides that oxidize or hydroxylate a retinoid or retinol or retinoic acid or all-*trans* retinoic acid at any position. Therefore, they encompass a genus of polypeptides described by broad function. The specification teaches only one species of the claimed genus, an all-*trans* retinoic acid hydroxylase that specifically hydroxylates all-*trans* retinoic acid at the C4-and the C18-position of the β-ionone ring and oxidizes all-*trans* retinoic acid at the C4-position of the β-ionone ring. The specification does not teach polypeptide species with any other retinoid oxidizing/hydroxylating activity, i.e, said activity with substrate specificity as broad as any retinoid, i.e., retinoic acid (RA), retinal, retinol in every stereo configuration as well as undescribed natural and artificial variants thereof (see the specification, paragraph bridging pages 5 and 6).

Furthermore, according to the definition of "retinoid", the genus of retinoids encompasses "a group of compounds which includes retinoic acid, vitamin A (retinol) and a series of natural and synthetic derivatives that an exert profound effects on development and differentiation in a wide variety of systems" (specification, paragraph bridging pages 4 and 5). The specification does not discloses any species within the genus of retinoids other than naturally occurring retinoic acid and derivatives thereof. There is no description of any synthetic derivative thereof or any natural or synthetic compound that can be construed as exerting profound effects on development and differentiation in a wide variety of systems. Therefore, the genus of retinoids

encompasses compounds of different structures and functions and the specification fails to disclose the correlation between structure and function common to all members of the genus.

In addition, claims 97-102 and 104-106 are drawn to a polypeptide that binds to an antibody specific for SEQ ID NOs: 2, 4 or 32 or is encoded by a DNA that hybridizes to the DNA encoding thereof and has no known function.

Therefore, the claims are drawn to a genus of polypeptides of undefined structures having any function.

The genus of polypeptides that comprises these above polypeptide molecules is a large variable genus encompassing many different proteins and fragments thereof. Many structurally and functionally unrelated polypeptides are encompassed within the scope of these claims, including partial sequences. Said genus encompasses both polypeptides having an enzymatic activity and inactive variants thereof as well polypeptides with undisclosed function. The specification fails to provide identifying characteristics and/or correlation between structure and function common to all members of the genus.

Furthermore, claims 85 and 92 are drawn to polypeptides encoded by nucleotide sequ nces that are part of fish genome. This part of rejection comprises polypeptides with a narrow function such as a retinoic acid inducible polypeptides having all-*trans*

retinoic acid 4-hydroxylase. The specification teaches only a single species of the claimed diverse genus, an all-*trans* retinoic acid 4-hydroxylase from zebrafish. The recitation of "fish genome" fails to provide a sufficient description of the claimed genus of proteins as it merely describes the functional features of the genus without providing any definition of the structural features of the species within the genus. The CAFC in *UC California v. Eli Lilly*, (43 USPQ2d 1398) stated that: "In claims to genetic material, however a generic statement such as "vertebrate insulin cDNA" or "mammalian insulin cDNA", without more, is not an adequate written description of the genus because it does not distinguish the claimed genus from others, except by function. It does not specifically define any of the genes that fall within its definition. It does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus". Similarly with the claimed genus of proteins the functional definition of the genus does not provide any structural information commonly possessed by members of the genus which distinguish the protein species within the genus from other proteins such that one can visualize or recognize the identity of the members of the genus.

Thus, a polypeptide other than a polypeptide having 1) all-*trans* retinoic acid 4-hydroxylase activity and 2) an amino acid sequence of SEQ ID NO: 2, 4 or 32 or that is encoded by a DNA that hybridizes under highly stringent conditions to SEQ ID NO:3, 5

or 31 lacks sufficient written description needed to practice the invention of claims 83-95, 97-102 and 104-112.

Claims 83, 85-90, 92-95, 97-102 and 104-112 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a all-*trans* retinoic acid 4-hydroxylase encoded by SEQ ID NOs: 3, 5 or 31 or encoded by a sequence that hybridizes thereto under highly stringent conditions, does not reasonably provide enablement for a conservatively substituted amino acid variant thereof, a retinoid oxidase of any substrate specificity that is encoded by a sequence that hybridizes to SEQ ID NOs: 3, 5 or 31 or a degenerate variant thereof and a conservatively substituted amino acid variant thereof as well as a polypeptide that oxidizes/hydroxylates retinoid or having unknown function that binds to an antibody specific for SEQ ID NOs: 3, 5 or 31. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The claims are broader than the enablement provided by the disclosure with regard to the huge number of all possible derivatives having the desired enzymatic activities.

Factors to b considered in determining whether undue experimentation is required, are summarized in In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir.

1988). They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

Factors pertinent to this discussion include predictability of the art, guidance in the specification, breadth of claims, and the amount of experimentation that would be necessary to use the invention.

Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function.

While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claim, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any prot in and the result of such modifications is

unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass all modifications and fragments of any sequence that is claimed in claims 83, 85-90, 92-95, 97-102 and 104-112 because the specification does **not** establish: (A) regions of the protein structure which may be modified without effecting the requisite activity. The specification does not teach the structure that is responsible for a specific all-*trans* retinoic acid 4-hydroxylase activity as compared to any other retinoid oxidizing activity;(B) the general tolerance of a protein to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any amino acid residues with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful. This reasoning is also applicable to cytochrome P450 structures comprising the heme binding region because there is no guidance provided as to what are residues that are responsible for the substrate specificity of the enzymes of the instant invention.

Furthermore, degenerate variants of SEQ ID NOs: 3, 5 or 31 encompass an enormous number of molecules. The specification does not provide any guidance as to

which of these degenerate variants can be used, so that a DNA that hybridizes thereto would encode a protein with the requisite properties.

With regard to claims 85 and 92, the rejection applies because the specification enables only for polypeptides that are encoded by DNAs that hybridize to SEQ ID NO: 3, 5 or 31 under highly stringent conditions, it does teach how to obtain variant polypeptides of unknown homology to SEQ ID NO:2, 4 or 32.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including a number of amino acid modifications of SEQ ID NOs: 2, 4 or 32. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of a protein having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue.

Claims 97-102 and 104-106 are drawn to proteins with no function. Applicants have not provided sufficient guidance as to what is the function of proteins encompassed by the claims.

The state of the art does not allow the predictability of the properties based on the structure. Therefore, one skilled in the art would require guidance as to how to use a polypeptide of unknown function that binds to an antibody specific for SEQ ID NOs: 2,

4 or 32 or is encoded by a DNA that hybridizes to a DNA encoding thereof in a manner reasonably correlated with the scope of the claims. Without such guidance, the experimentation left to those skilled in the art is undue.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 83-95, 97-102 and 104-112 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 83-90, 92-95 recite "conservatively substituted amino acid variant thereof". There are no clear assigned definitions of the term "conservatively substituted amino acid variant" in the art.

Claims 97-102 and 104-112 are confusing because they recite an antibody that is elicited by SEQ ID NO:2, 4 or 32 and by an epitope of unknown structure.

Claims 83-86, 89-92, 95 and 107-112 recite "retinoid". This term is defined as comprising "a series of natural and synthetic derivatives" (emphasis added). The metes and bonds of "derivative" are neither disclosed in the specification nor clearly known in the art.

Art Unit: 1652

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 83-95, 97-102 and 104-112 are rejected under 35 U.S.C. 102(b) as being anticipated by Duell et al. (1992).

Duell et al. (form PTO-1449 filed February 6, 2003, reference 6, J. Clin. Investigation (1992), 90,1269-1274) teach a retinoic acid induced all-*trans* retinoic acid 4-hydroxylase activity in human skin microsomes. They teach that said activity catalyzes conversion of RA to 4-OH RA and 4-oxo RA (abstract, page 1271).

The microsomal fraction having the requisite activity contains a polypeptide with said activity. Said peptide is "isolated" from its natural environment. Therefore, the Duell et al. reference anticipates claims 83-95, 97-102 and 104-112.

Claims 83-95, 97-102 and 104-112 are rejected under 35 U.S.C. 102(b) as being anticipated by Duell et al. (1994).

Duell et al. (form PTO-1449 filed February 6, 2003, referenc 6, J. Investigative Dermatology (1994), Vol. 102, page 641, SID abstracts, abstract 704) teach a unique

cytochrome P450 that has retinoic acid induced all-*trans* retinoic acid 4-hydroxylase activity but does not metabolize 9-cis or 13-cis retinoic acid. They demonstrated said activity in human skin microsomes.

The microsomal fraction having the requisite activity contains a polypeptide with said activity. Said peptide is "isolated" from its natural environment. Therefore, the Duell et al. reference anticipates claims 83-95, 97-102 and 104-112.

Claims 97, 99, 101 and 104-112 are rejected under 35 U.S.C. 102(b) as being anticipated by Vetter et al.

Vetter et al. teach the amino acid sequence of an inducible cytochrome P-450 protein from Periwinkle (*Catharanthus roseus* L.) (page 1002, Figure 3.). Since an epitope is not limited to a specific fragment, this polypeptide will bind to an antibody elicited by an epitope of some five, for example, amino acids within the conserved region of SEQ ID NOs: 2, 4 or 32 and, therefore anticipates claims 97, 99, 101 and 104-112.

Claims 97, 99, 101 and 104-112 are rejected under 35 U.S.C. 102(b) as being anticipated by Shen et al.

Shen et al. teach the amino acid sequence of a mouse cytochrome P-450 protein (page 11485, Figure 3.). Since an epitope is not limited to a specific fragment,

this polypeptide will bind to an antibody elicited by an epitope of some five, for example, amino acids within the conserved region of SEQ ID NOs: 2, 4 or 32 and, therefore anticipates claims 97, 99, 101 and 104-112.

Response to Arguments

Applicant's arguments filed May 31, 2002 have been fully considered but they are not persuasive.

Applicants argue that claims 97-102 and 104-112 do not introduce the new matter because the specification on page 36 teaches that "antibodies can be used to detect the proteins of the invention, portions thereof or closely related isoforms in various biological materials" (page 10). This is not persuasive because there is support for an antibody and a method of use thereof but not for proteins that can be detected using said antibodies.

With regard to conservative variants, applicants disagree that they amount to any protein having the desired function (pages 11-12). Applicants assert that "it is only conservatively substituted variants of these proteins that are claimed. The claim includes only those variant proteins in which an individual amino acid is substituted for another amino acid of protein to have function. ... while it is admitted there is no limitation on the number of such substitutions that might be made, any such substitution is clearly based on one of basic claimed structures(a protein encoded by the sequence

that hybridizes to SEQ ID NO:3, 5, etc.) and would be known to the skilled person" (page 12, 2nd paragraph). This is not persuasive because claims are not drawn to proteins having substitutions from other proteins. Ad in any event, there is no limitation on the number and location of residues to be substituted.

Applicants further argue with regard to the function that "applicants reasonable expect that P450RAI oxidizes the 18 position of the β-ionone ring as well as the 4 position, and that P450RAI oxidizes retinol as well as RA (see page 3, lines 23-26)" (page 13, 2nd paragraph). The specification on page 3, lines 23-26 reads differently. However, on page 3, lines 27-30, it provides support for the 4 position of β-ionone ring of RA. However, the claims recite much broader specificity expanding the scope of the claims to encompass retinoid oxidizing enzymes of different function and properties. With regard to the 102 rejection, applicants argue that "the antibody is elicited by an epitope located within a specified unconserved region of the protein" (page 17). This is not persuasive because within this region the proteins of the instant invention share a few identical or conservatively substituted amino acids with the proteins of the references. An antibody elicited by such fragment would cross react with many proteins including the proteins of the instant invention and the references.

Conclusion

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

Frolik et al. (1979, form PTO-1449 filed February 6, 2003, reference 8) teach all-*trans* retinoic acid 4-hydroxylase activity in hamster trachea and liver.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Elizabeth Slobodyansky whose telephone number is (703) 306-3222. The examiner can normally be reached Monday through Friday from 9:30 AM to 6:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Ponnathapura Achutamurthy, can be reached at (703) 308-3804. The FAX phone number for Technology Center 1600 is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Center receptionist whose telephone number is (703) 308-0196.

E. Slobodyansky

Elizabeth Slobodyansky, PhD
Primary Examiner

February 12, 2003

Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet	1	of	1

Complete if Known	
Application Number	09/668,482
Filing Date	September 25, 2000
First named Inventor	PETKOVICH
Group Art Unit	1652
Examiner Name	SLOBODYANSKY, E.
Atty Docket Number	57600/00035

RECEIVED 03 FEB 16 TECH CENTER 1600/2900

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	U.S. Patent Document Number	Kind Code[2] (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Office[3]	Number[4]	Kind Code[5] (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
ES	A1	WO	WO93/22331		ASTROM et al.	11-11-1993		
ES	A2	WO	WO92/16658		MANGELSDORF et al.	10-01-1992		
ES	A3	WO	WO96/23080		NAGPAL et al.	08-01-1996		
ES	A4	EP	0 721 984 A1		COLLEN et al.	07-17-1996		

Examiner Signature	E. Slobodyansky	Date Considered	2/10/03

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 1 of 5

Complete if Known	
Application Number	09/668,462
Filing Dat	September 25, 2000
First named Inventor	PETKOVICH
Group Art Unit	1652
Examiner Name	SLOBODYANSKY, E.
Atty Docket Number	57600/00035

OTHER DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, page(s), volume-issue number(s) publisher, city and/or country where published	T[2]
ES	1	ADAMSON P.C. et al., "Time Course of Induction of Metabolism..." Cancer Research, 53: 472-476 (1993).	
	2	BOYLAN J.F. et al., "Targeted Disruption of Retinoic Acid Receptor..." Molecular and Cellular Biology, 15: 2, 843-851 (Feb. 1995).	
	3	DE COSTER R. et al., "Experimental Studies with Liarozole..." J. Steroid Molec. Biol., 43: 1-3, 197-201 (1992).	
	4	FIORELLA, P.D. et al., "Microsomal Retinoic Acid Metabolism..." Journal of Biological Chemistry, 269: 14, 10538-10544 (April 1994).	
	5	DENISON, M.S. et al., "Xenobiotic-inducible transcription of cytochrome P450 genes", J. Biol. Chem., 270: 18175-18178 (1995).	
	6	DUELL, E.A. et al., "Human skin levels of retinoic acid and cytochrome P-450-derived 4-hydroxyretinoic acid after topical application of retinoic acid in vivo compared to concentrations required to stimulate retinoic acid receptor-mediated transcription in vitro", J. Clin. Invest., 90: 1269-1274 (1992).	
	7	DUELL, E.A. et al., "All-trans, 9-cis and 13-cis retinoic acid each induce a cytochrome P450 4-retinoic acid hydroxylase which causes all-trans but not 9-cis or 13-cis retinoic acid to self-metabolize", SID Abstracts, 102: 641 (1994). Experimental Dermatology	
	8	FROLIK, C.A., et al., "Isolation and identification of 4-hydroxy- and 4-oxoretinoic acid. In vitro metabolites of all-trans-retinoic acid in hamster trachea and liver", Biochemistry, 18: 2092-2097 (1979).	
	9	MUINDI, J.R.F. et al., "Clinical pharmacology of all-trans retinoic acid", Leukemia, 8: S16-S21 (1994).	
	10	ROBERTS, A.B. et al., "In vitro metabolism of retinoic acid in hamster intestine and liver", J. Biol. Chem., 254: 6296-6302 (1979).	
	11	ROBERTS, A.B. et al., "Retinoid-dependent induction of the in vivo and in vitro metabolism of retinoic acid in tissues of the vitamin A-deficient hamster", J. Biol. Chem., 254: 6303-6309 (1979).	
	12	TAKATSUKA, J. et al., "Retinoic acid metabolism and inhibition of cell proliferation: an unexpected liaison", Cancer Res., 56: 675-678 (1996).	
	13	VAN WAUWE, J.P., et al., "Ketoconazole inhibits the in vitro and in vivo metabolism of all-trans-retinoic acid", J. Pharm. Exp. Ther. 245: 718-722 (1988).	
	14	VAN WAUWE, J.P. et al., "Effects of cytochrome P-450 inhibitors on the in vivo metabolism of all-trans-retinoic acid in rats", J. Pharm. Exp. Ther. 252: 365-369 (1990).	
ES	15	VAN WAUWE, J.P. et al., "Liarozole, an inhibitor of retinoic acid metabolism, exerts retinoid-mimetic effects in vivo", J. Pharm. Exp. Ther. 261: 773-779 (1992).	

Examiner Signature	E. Slobodyansky	Date Considered	2/10/03

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[2] if English language Translation is attached.

Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sh et 2 of 5

Complete if Known	
Application Number	09/668,482
Filing Dat	September 25, 2000
First named Inventor	PETKOVICH
Group Art Unit	1652
Examiner Name	SLOBODYANSKY, E.
Atty Docket Number	57600/00035

OTHER DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, page(s), volume-issue number(s) publisher, city and/or country where published	T[2]
ES	16	WILLIAMS, J.B., et al., "Inhibition of retinoic acid metabolism by imidazole antimycotics in F9 embryonal carcinoma cells", Biochem. Pharm. 36: 1386-1388 (1987).	
	17	WOUTERS, W., et al., "Effects of liarozole, a new antitumoral compound, on retinoic acid-induced inhibition of cell growth and on retinoic acid metabolism in MCF-7 human breast cancer cells", Cancer Res. 52: 2841-2846 (1992).	
	18	ACHKAR, C.C. et al., "4-Oxoretinol, a new natural ligand and transactivator of the retinoic acid receptors", Proc. Natl. Acad. Sci. USA, 93: 4879-4884 (1996).	
	19	AKIMENKO, M.-A. et al., "Anterior duplication of the Sonic hedgehog expression pattern in the pectoral fin buds of zebrafish treated with retinoic acid", Dev. Biol., 170: 243-247 (1995).	
	20	AKIMENKO, M.-A. et al., "Differential induction of four msx homeobox genes during fin development and regeneration in zebrafish", Development, 121: 347-357 (1995).	
	21	AKIYOSHI-SHIBATA, M. et al., "Further oxidization of hydroxycalcidiol by calcidiol 24-hydroxylase. A study with the mature enzyme expressed in Escherichia coli", Eur. J. Biochem., 224: 335-343 (1994).	
	22	BLUMBERG, B. et al., "Novel retinoic acid receptor ligands in Xenopus embryos", Proc. Natl. Acad. Sci. USA, 93: 4873-4878 (1996).	
	23	BOYLE, A.L. et al., "Rapid physical mapping of cloned DNA on banded mouse chromosomes by fluorescence in situ hybridization", Genomics 12: 106-115 (1992).	
	24	CASTONGUAY, A. et al., "Expression of xenobiotic-metabolizing enzymes in cultured rat tracheal epithelial cells", Environ. Health Perspect. 103: 254-258 (1995).	
	25	CHAMBON, P., "The molecular and genetic dissection of the retinoid signaling pathway", Recent Progress in Hormone Research, 50: 317-332 (1995).	
	26	CHEN, K.-S., et al., "Cloning of the human 1 α,25-dihydroxyvitamin D-3 24-hydroxylase gene promoter and identification of two vitamin D-responsive elements", Biochim. Biophys. Acta, 1263: 1-9 (1995).	
	27	COSTARIDIS, P. et al., "Endogenous retinoids in the zebrafish embryo and adult", Dev. Dynamics, 205: 41-51 (1996).	
	28	CREECH KRAFT, J. et al., "Temporal distribution, localization and metabolism of all-trans-retinol, didehydroretinol and all-trans-retinal during Xenopus development", Biochem J., 301: 111-119 (1994).	
ES	29	FIORELLA, P.D. et al., "Expression of cellular retinoic acid-binding protein (Type II) in Escherichia coli", J. Biol. Chem., 268: 21545-21552 (1993).	

Examiner Signature	E. Slobodyansky	Date Considered	2/10/03

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

 if English language Translation is attached.

Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet	3	of	5

Complete if Known	
Application Number	09/668,482
Filing Date	September 25, 2000
First named Inventor	PETKOVICH
Group Art Unit	1652
Examiner Name	SLOBODYANSKY, E.
Atty Docket Number	57600/00035

OTHER DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, page(s), volume-issue number(s) publisher, city and/or country where published	T[2]
EG	30	GREEN, S., et al., "A versatile in vivo and in vitro eukaryotic expression sector for protein engineering", Nucl. Acids Res., 16: 369 (1988).	
	31	GUBLER, M.L. et al., "Metabolism of Retinoic Acid and Retinol..." Methods in Enzymology, 189, 525-530 (1990).	
	32	GUENGERICH, F.P., "Reactions and significance of cytochrome P-450 enzymes", J. Biol. Chem., 266: 10019-10022 (1991).	
	33	HAN, I.S. et al., "Highly specific cytochrome P450-like enzymes for all-trans-retinoic acid in T47D human breast cancer cells", J. Clin. Endocr. Metab. 81: 2069-2075 (1996).	
	34	HENG, H.H.Q. et al., "Modes of DAPI banding and simultaneous in situ hybridization", Chromosoma, 102: 325-332 (1993).	
	35	HILLIER, et al., Accession R51129, EST-STS, EST-STS-Two databases (May, 1995).	
	36	JONES, B.B. et al., "New retinoid X receptor subtypes in zebra fish (Danio rerio) differentially modulate transcription and do not bind 9-cis retinoic acid", Mol. Cell. Biol., 15: 5226-5234 (1995).	
	37	LAMMER, E.J. et al., "Retinoic acid embryopathy", N. Engl. J. Med., 313: 837-841 (1985).	
	38	LEO, M.A. et al., "Metabolism of retinol and retinoic acid by human liver cytochrome P450IIC8", Arch. Biochem. Biophys. 269: 305-312 (1989).	
	39	LEO, M.A. et al., "Retinoic acid metabolism by a system reconstituted with cytochrome P-450", Arch. Biochem. Biophys. 234: 305-312 (1984).	
	40	LIANG, P. et al., "Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction", Science, 257: 967-971 (1992).	
	41	LICHTER, P. et al., "High-resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones", Science, 247: 64-69 (1990).	
	42	MADEN, M. et al., "Retinoic acid and development of the central nervous system", BioEssays, 14: 431-438 (1992).	
	43	MAKIN, G. et al., "Target cell metabolism of 1,25-dihydroxyvitamin D3 to calcitroic acid", Biochem. J., 262: 173-180 (1989).	
	44	MANGELSDORF, D.J. et al., "The RXR heterodimers and orphan receptors", Cell 83: 841-850 (1995).	
EG	45	MARTINI, R. et al., "Participation of P450 3A enzymes in rat hepatic microsomal retinoic acid 4-hydroxylation", Arch. Biochem. Biophys. 303: 57-66 (1993).	

Examiner Signature	E. Slobodyansky	Date Considered	2/10/03

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1]Unique citation designation number. [2]Applicant is to place a check mark here if English language Translation is attached.

Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 4 of 5

Complete If Known	
Application Number	09/668,482
Filing Dat	September 25, 2000
First named Inventor	PETKOVICH
Group Art Unit	1652
Examiner Name	SLOBODYANSKY, E.
Atty Docket Number	57600/00035

OTHER DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, page(s), volume-issue number(s) publisher, city and/or country where published	T[2]
ES	46	MARTINI, R. et al., "Retinal dehydrogenation and retinoic..." Biochemical Pharmacology, 47, No. 5, 905-909, 1994.	
	47	MARIKAR, Y. et al., "Regulation, properties, and solubilization of a unique cytochrome P-450 that specifically metabolizes all-trans retinoic acid to less active 4-hydroxy retinoic acid in human keratinocyte HACAT cells", Abstract, J. Invest. Dermatol. 106(4): 807 (1996).	
	48	MONIA, B.P. et al., "Antitumor activity of a phosphorothioate antisense oligodeoxynucleotide targeted against C-raf kinase", Nature Med., 2: 668-675 (1996).	
	49	MORRISS-KAY, G., "Retinoic acid and craniofacial development: molecules and morphogenesis", BioEssays, 15: 9-15 (1993).	
	50	MUINDI, J.R.F. et al., "Clinical pharmacology of oral all-trans retinoic acid in patients with acute promyelocytic leukemia", Cancer Res., 52: 2138-2142 (1992).	
	51	MUINDI, J.R.F., et al., "Climical pharmacology of all-trans retinoic Acid" Liekemis 8:1807-1812 (1994).	
	52	MUINDI, J.F. et al., "Lipid Hydroperoxides Greatly Increase the Rate..." Cancer Research, 53, 1226-1229 (March 1993).	
	53	MURTHA, M.L. et al., "Detection of homeobox genes in development and evolution", Proc. Natl. Acad. Sci. USA, 88: 10711-10715 (1991).	
	54	NAPOLI, J.L. et al., "The biosynthesis of retinoic acid from retinol by rat tissues in vitro", Arch. Biochem. Biophys., 255: 95-101 (1987).	
	55	NELSON, D.R. et al., "The P450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature", DNA and Cell Biol., 12: 1-51 (1993).	
	56	OGURA, T. et al., "A retinoic acid-triggered cascade of HOXB1 gene activation", Proc. Natl. Acad. Sci. USA 92: 387-391(1995).	
	57	OHYAMA, Y. et al., "Identification of a vitamin D-responsive element in the 5'-flanking region of the rat 25-hydroxyvitamin D3 24-hydroxylase gene", J. Biol. Chem., 269: 10545-10550 (1994).	
	58	PIJNAPPEL, W.W.M. et al., "The retinoid ligand 4-oxo-retinoic acid is a highly active modulator of positional specificiation", Nature, 366: 340-344 (1993).	
ES	59	RANER, G.M. et al., "Metabolism of all-trans, 9-cis, and 13-cis isomers of retinal by purified isozymes of microsomal cytochrome P450 and mechanism-based inhibition of retinoid oxidation by citral", Mol. Pharmacol. 49: 515-522 (1996).	

Examiner Signature	E. Slobodyansky	Date Considered	2/10/03

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1]Unique citation designation number. [2]Applicant is to place a check mark here if English language Translation is attached.

Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet	5	of	5

Complete if Known	
Application Number	09/668,482
Filing Date	September 25, 2000
First named Inventor	PETKOVICH
Group Art Unit	1652
Examiner Name	SLOBODYANSKY, E.
Atty Docket Number	57600/00035

OTHER DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, page(s), volume-issue number(s) publisher, city and/or country where published	T[2]
ES	60	REDDY, A.P. et al., "Characterization and purification of human retinoic acid receptor-γ1 overexpressed in the baculovirus-insect cell system", Biochem J., 287: 833-840 (1992).	
	61	THALLER, C., et al., "Isolation of 3,4-didehydroretinoic acid, a novel morphogenetic signal in the chick wing bud", Nature, 345: 815-819 (1990).	
	62	TOMITA, S., et al., "Characteristic properties of a retinoic acid synthetic cytochrome P-450 purified from liver microsomes of 3-methylcholanthrene-induced rats", Biochim. Biophys. Acta 1290: 273-281 (1996).	
	63	WHITE, J.A., et al., "A zebrafish retinoic acid receptor expressed in the regenerating caudal fin", Development,120: 1861-1872 (1994).	
	64	WHITE, J.A., et al., "Identification of the retinoic acid-inducible all-trans-retinoic acid 4-hydroxylase", J. Biol. Chem. 271: 29922-29927 (1996).	
	65	WINDHORST, D.B., "The use of isotretinoin in disorders of keratinization", J. Am. Acad. Dermatol. 6: 708-709 (1982).	
ES	66	ZIEROLD, C. et al., "Two vitamin D response elements function in the rat 1,25-dihydroxyvitamin D 24-hydroxylase promoter", J. Biol. Chem., 270: 1675-1678 (1995)	

Examiner Signature	E. Slobodyansky	Date Considered	2/10/03

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1]Unique citation designation number. [2]Applicant is to place a check mark here if English language Translation is attached.

Exhibit B

Page 1 of 29 pages

PTO/SB/21 (01-03)
Approved for use through 04/30/2003. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 279 / 28

Application Number	09/668,482
Filing Date	09/25/2000
First Named Inventor	PETKOVICH, Martin P.
Art Unit	1652
Examiner Name	SLOBODYANSKY, Elizabeth
Attorney Docket Number	32391-2005

ENCLOSURES (Check all that apply)

- [X] Fee Transmittal Form
- [X] Fee Attached
- [X] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [X] Extension of Time Request
- [ ] Express Abandonment Request
- [X] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Response to Missing Parts/ Incomplete Application
  - [ ] Response to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation
- [ ] Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
- [ ] After Allowance Communication to Group
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to Group (Appeal Notice, Brief, Reply Brief)
- [ ] Proprietary Information
- [ ] Status Letter
- [X] Other Enclosure(s) (please identify below): Letter under 37 CFR 1.56

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual	John Hunt, Registration No. 36,424
Signature	John Hunt
Date	08/11/2003

CERTIFICATE OF TRANSMISSION/MAILING - To Fax. 308. 4242

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, Washington, DC 20231 on this date: 08/11/2003

Typed or printed	John Hunt		
Signature	John Hunt	Date	08/11/2003

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, Washington, DC 20231.

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

PTO/SB/21 (01-03)
Approved for use through 04/30/2003. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 276 / 36

Application Number	09/668,482
Filing Date	09/25/2000
First Named Inventor	PETKOVICH, Martin P.
Art Unit	1652
Examiner Name	SLOBODYANSKY, Elizabeth
Attorney Docket Number	32391-2005

ENCLOSURES *(Check all that apply)*

- [X] Fee Transmittal Form
- [X] Fee Attached
- [X] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [X] Extension of Time Request
- [ ] Express Abandonment Request
- [X] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Response to Missing Parts/ Incomplete Application
  - [ ] Response to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
- [ ] After Allowance Communication to Group
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to Group (Appeal Notice, Brief, Reply Brief)
- [ ] Proprietary Information
- [ ] Status Letter
- [X] Other Enclosure(s) (please identify below): Letter under 37 CFR 1.56

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual	John Hunt, Registration No. 36,424
Signature	John Hunt
Date	08/11/2003

CERTIFICATE OF TRANSMISSION/MAILING To 703. 308. 4242

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, Washington, DC 20231 on this date: 08/11/2003

Typed or printed	John Hunt		
Signature	John Hunt	Date	08/11/2003

PTO/SB/17 (01-03)
Approved for use through 04/30/2003. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

FEE TRANSMITTAL for FY 2003

Effective 01/01/2003. Patent fees are subject to annual revision.

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 1,110.00

Complete if Known	
Application Number	09/668,482
Filing Date	09/25/2000
First Named Inventor	PETKOVICH, Martin P.
Examiner Name	SLOBODYANSKY, Elizabeth
Art Unit	1652
Attorney Docket No.	32391-2005

METHOD OF PAYMENT *(check all that apply)*

☐ Check ☒ Credit card ☐ Money Order ☐ Other ☐ None

☐ Deposit Account:

Deposit Account Number

Deposit Account Name

The Commissioner is authorized to: *(check all that apply)*

☐ Charge fee(s) indicated below ☒ Credit any overpayments

☒ Charge any additional fee(s) during the pendency of this application

☐ Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	

SUBTOTAL (1) ($) 0.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

		Extra Claims		Fee from below		Fee Paid
Total Claims	-20** =	0	X		=	$0
Independent Claims	- 3** =	0	X		=	$0
Multiple Dependent					=	$0

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0.00

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	*For filing a request for ex parte reexamination*	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	$930.00
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing a brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,300	2453	650	Petition to revive - unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	$180.00
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($) 1,110.00

SUBMITTED BY				(Complete if applicable)	
Name (Print/Type)	John Hunt	Registration No. (Attorney/Agent)	36,424	Telephone	416.865.8121
Signature				Date	08/11/2003

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

This collection of information is required by 37 CFR 1.17 and 1.27. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO:

PTO-2038 (02-2000)
Approved for use through 01/31/2003. OMB 0651-0043
United States Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number

United States Patent & Trademark Office

Credit Card Payment Form
Please Read Instructions before Completing this Form

Credit Card Information

Credit Card Type:	(Visa)	MasterCard	American Express	Discover

Credit Card Account#: 4520 7000 0112 1167

Credit Card Expiration Date: 10/04

Name as It Appears on Credit Card: Torys LLP Robin Coster

Payment Amount: $(US Dollars): 1,110.00

Signature: [signature] | Date:AUGUST 11, 2003

Refund Policy: The Office may refund a fee paid by mistake or in excess of that required. A change of purpose after the payment of a fee will not entitle a party to a refund of such fee. The Office will not refund amounts of twenty-five dollars or less unless a refund is specifically requested, and will not notify the payor of such amounts (37 CFR 1.26). Refund of a fee paid by credit card will be via credit to the credit card account.
Service Charge: There is a 50.00 service charge for processing each payment refused (including a check returned "unpaid") or charged back by a financial institution (37 CFR 1.21(m)).

Credit Card Billing Address

Street Address 1: 3000 – 79 Wellington Street West

Street Address 2: Box 270, TD Centre

City: Toronto

State: Ontario | Zip/Postal Code: M5K 1N2

Country: Canada

Daytime Phone #: 416 865 7228 | Fax #: 416 865 7380

Request and Payment Information

Description of Request and Payment Information 1. Extension Fee (3 month) and 2. Submission of IDS

Patent Fee	Patent Maintenance Fee	Trademark Fee	Other Fee
Application No.09/668,482	Application No.	Serial No.	IDON Customer No.
Patent No.	Patent No.	Registration No.	
Attorney Docket No. 32391-2005		Identity or Describe Mark	

PTO/SB/22 (08-03)
Approved for use through 7/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARMENT OF COMMERCE

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)	Docket Number (Optional) 32391-2005

In re Application of

Application Number 09/668,482	Filed 09/25/2000
For RETINOID METABOLIZING PROTEIN	
Art Unit 1652	Examiner SLOBODYANSKY, E.

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and appropriate non-small-entity fee are as follows (check time period desired):

- ☐ One month (37 CFR 1.17(a)(1)) $_____
- ☐ Two months (37 CFR 1.17(a)(2)) $_____
- ☒ Three months (37 CFR 1.17(a)(3)) $930.00
- ☐ Four months (37 CFR 1.17(a)(4)) $_____
- ☐ Five months (37 CFR 1.17(a)(5)) $_____

☐ Applicant claims small entity status. See 37 CFR 1.27. Therefore, the fee amount shown above is reduced by one-half, and the resulting fee is: $_____.

☐ A check in the amount of the fee is enclosed.

☒ Payment by credit card. Form PTO-2038 is attached.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 502651.

I have enclosed a duplicate copy of this sheet.

I am the ☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed (Form PTO/SB/96).

☒ attorney or agent of record. Registration Number 36,424

☐ attorney or agent under 37 CFR 1.34(a).
Registration number if acting under 37 CFR 1.34(a) _____.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

August 11, 2003
Date


Signature

416.865.8121
Telephone Number

John C. Hunt
Typed or printed name

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

☐ Total of _____ forms are submitted.

PTO/SB/22 (08-03)
Approved for use through 7/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARMENT OF COMMERCE

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a) | Docket Number (Optional) 32391-2005

In re Application of

Application Number 09/668,482 | Filed 09/25/2000

For RETINOID METABOLIZING PROTEIN

Art Unit 1652 | Examiner SLOBODYANSKY, E.

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.

The requested extension and appropriate non-small-entity fee are as follows (check time period desired):

- ☐ One month (37 CFR 1.17(a)(1)) $_____
- ☐ Two months (37 CFR 1.17(a)(2)) $_____
- ☒ Three months (37 CFR 1.17(a)(3)) $930.00
- ☐ Four months (37 CFR 1.17(a)(4)) $_____
- ☐ Five months (37 CFR 1.17(a)(5)) $_____

☐ Applicant claims small entity status. See 37 CFR 1.27. Therefore, the fee amount shown above is reduced by one-half, and the resulting fee is: $_____.

☐ A check in the amount of the fee is enclosed.

☒ Payment by credit card. Form PTO-2038 is attached.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 502651.

I have enclosed a duplicate copy of this sheet.

I am the ☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed (Form PTO/SB/96).

☒ attorney or agent of record. Registration Number 36,424

☐ attorney or agent under 37 CFR 1.34(a). Registration number if acting under 37 CFR 1.34(a) _____.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

August 11, 2003 — Date


Signature

416.865.8121 — Telephone Number

John C. Hunt — Typed or printed name

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

☐ Total of _____ forms are submitted.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	Petkovich *et al.*
Serial No.:	09/668,482
Filing Date:	September 25, 2000
Title:	Retinoid Metabolizing Protein
Art Unit:	1652
Examiner:	Elizabeth Slobodyansky, Ph.D.
Atty's Docket No.:	32391-2005

The Commissioner of Patents and Trademarks
Washington, D.C. 20331
U.S.A.

AMENDMENT

Dear Sir:

This is a response to the non-final office action of February 13, 2003.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

LISTING OF CLAIMS

Claims 1 to 82 (previously cancelled)

83. (Currently amended) **[An isolated]** **A microsomal preparation comprising a recombinant** protein **expressed by a cell that has been transfected with a nucleic acid molecule encoding the protein, or by a descendent cell thereof, [which] wherein said protein** oxidizes **[a retinoid]** **all-*trans* retinoic acid at the C4-position of the ß-ionone ring, said [and encoded by a]** nucleic acid molecule comprising a nucleotide sequence that hybridizes under high stringency conditions, wherein high stringency conditions include a wash step of about 0.2 x SSC at 50°C, to a polynucleotide having a nucleotide sequence selected from the group of sequences shown as: SEQ ID NO:3; **[a sequence which varies from SEQ ID NO:3 in a coding region due to the degeneracy of the genetic code;]** SEQ ID NO:5; **[a sequence which varies from SEQ ID NO:5 in a coding region due to the degeneracy of the genetic code;]** **and** SEQ ID NO:31; **[a sequence which varies from SEQ ID NO:31 in a coding region due to the degeneracy of the genetic code, or a conservatively substituted amino acid variant of a said protein which oxidizes a retinoid] and wherein the microsomal preparation is substantially free of other proteins that are cytochromes expressed by epidermal cells.**

Claims 84 to 89 (Cancelled)

90. (Currently amended) **[An isolated]** **A microsomal preparation comprising a recombinant** protein **expressed by a cell that has been transfected with a nucleic acid molecule encoding the protein, or by a descendent cell thereof, [which] wherein said protein** hydroxylates **[a retinoid]** **all-*trans* retinoic acid** at the **C4-position [4-position]** of the ß-ionone ring, **said [and encoded by a]** nucleic acid molecule comprising a nucleotide sequence that hybridizes under high stringency

conditions, wherein high stringency conditions include a wash step of about 0.2 x SSC at 50°C, to a nucleic acid molecule having a nucleotide sequence selected from the group of sequences shown as: SEQ ID NO:3; **[a sequence which varies from SEQ ID NO:3 in a coding region due to the degeneracy of the genetic code;]** SEQ ID NO:5; **[a sequence which varies from SEQ ID NO:5 in a coding region due to the degeneracy of the genetic code;]** and SEQ ID NO:31; **[a sequence which varies from SEQ ID NO:31 in a coding region due to the degeneracy of the genetic code, or a conservatively substituted amino acid variant of a said protein which hydroxylates a retinoid]** **and wherein the microsomal preparation is substantially free of other proteins that are cytochromes expressed by epidermal cells.**

Claims 91 to 112 (Cancelled)

113. (New) The preparation of claim 83, wherein said nucleotide sequence hybridizes under said conditions to SEQ ID NO:3.

114. (New) The preparation of claim 83, wherein said nucleotide sequence hybridizes under said conditions to SEQ ID NO:5.

115. (New) The preparation of claim 83, wherein said nucleotide sequence hybridizes under said conditions to SEQ ID NO:31.

116. (New) The preparation of claim 114, wherein the amino acid sequence identity between the protein and SEQ ID NO:4 is at least about 93 percent.

117. (New) The preparation of claim 116, wherein the protein comprises the amino acid sequence identified as SEQ ID NO:4.

118. (New) The preparation of claim 113, wherein the protein comprises the amino acid sequence identified as SEQ ID NO:2.

119. (New) The preparation of claim 115, wherein the protein comprises the amino acid sequence identified as SEQ ID NO:32.

120. (New) The preparation of claim 83, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 35 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

121. (New) The preparation of claim 120, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 40 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

122. (New) The preparation of claim 121, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 50 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

123. (New) The preparation of claim 122 wherein the nucleic acid molecule encodes an amino acid sequence that is at least 60 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

124. (New) The preparation of claim 123, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 65 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

125. (New) The preparation of claim 124, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 70 percent conserved with r spect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

126. (New) The preparation of claim 125, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 75 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

127. (New) The preparation of claim 126, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 85 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

128. (New) The preparation of claim 127, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 90 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

129. (New) The preparation of claim 128, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 95 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

130. (New) The preparation of claim 83, wherein the protein hydroxylates the C18-position of all-*trans* retinoic acid.

131. (New) The preparation of claim 90, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 35 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

132. (New) The preparation of claim 131, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 40 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

133. (New) The preparation of claim 132, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 50 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

134. (New) The preparation of claim 133, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 60 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

135. (New) The preparation of claim 134, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 65 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

136. (New) The preparation of claim 135, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 70 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

137. (New) The preparation of claim 136, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 75 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

138. (New) The preparation of claim 137, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 85 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

139. (New) The preparation of claim 138, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 90 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

140. (New) The preparation of claim 139, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 95 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

141. (New) The preparation of claim 90, wherein the protein hydroxylates the C18-position of all-*trans* retinoic acid.

142. (New) A microsomal preparation comprising a recombinant protein expressed by a cell that has been transfected with a nucleic acid molecule encoding the protein, or by a descendent cell thereof, wherein said protein oxidizes all-*trans* retinoic acid at the C4-position of the ß-ionone ring, said nucleic acid molecule encoding an amino acid sequence that is at least 60 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32, and wherein the microsomal preparation is substantially free of other proteins that are cytochromes expressed by epidermal cells.

143. (New) The preparation of claim 142, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 65 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

144. (New) The preparation of claim 143, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 70 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

145. (New) The preparation of claim 144, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 75 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

146. (New) The preparation of claim 145, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 85 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

147. (New) The preparation of claim 146, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 90 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

148. (New) The preparation of claim 147, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 95 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

149. (New) The preparation of claim 142, wherein the protein hydroxylates the C18-position of all-*trans* retinoic acid.

150. (New) A microsomal preparation comprising a recombinant protein expressed by a cell that has been transfected with a nucleic acid molecule encoding the protein, or by a descendent cell thereof, wherein said protein hydroxylates all-*trans* retinoic acid at the C4-position of the ß-ionone ring, said nucleic acid molecule encoding an amino acid sequence that is at least 60 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32, and wherein the microsomal preparation is substantially free of other proteins that are cytochromes expressed by epidermal cells.

151. (New) The preparation of claim 150, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 65 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

152. (New) The preparation of claim 151, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 70 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

153. (New) The preparation of claim 152, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 75 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

154. (New) The preparation of claim 153, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 85 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

155. (New) The preparation of claim 154, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 90 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

156. (New) The preparation of claim 155, wherein the nucleic acid molecule encodes an amino acid sequence that is at least 95 percent conserved with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32.

157. (New) The preparation of claim 150, wherein the protein hydroxylates the C18-position of all-*trans* retinoic acid.

158. (New) The preparation of claim 83, wherein the preparation is enriched at least 6.3 fold in said oxidase activity with respect to a microsomal preparation obtained from a non-transfected said cell under the same conditions.

159. (New) The preparation of claim 90, wherein the preparation is enriched at least 7.8 fold in said hydroxylase activity with respect to a microsomal preparation obtained from a non-transfected said cell under the same conditions.

160. (New) The preparation of claim 142, wherein the preparation is enriched at least 6.3 fold in said oxidase activity with respect to a microsomal preparation obtained from a non-transfected said cell under the same conditions.

161. (New) The preparation of claim 150, wherein the preparation is enriched at least 7.8 fold in said hydroxylase activity with respect to a microsomal preparation obtained from a non-transfected said cell under the same conditions.

REMARKS

Claims 83, 90 and 113 to 161 are pending in the application.

No new matter has been added by the amendments submitted herein, as explained further below.

Claim 83, as it previously read, was rejected under 35 U.S.C. § 112, first paragraph. Claim 83 has been amended to require the presence of a protein which oxidizes all-*trans* retinoic acid at the C4-position of the ß-ionone ring, as suggested in the first paragraph on page 5 of the outstanding Examiner's Report. Applicant believes this meets all concerns raised with respect to this claim under 35 U.S.C. § 112, first paragraph.

Claim 83, as it read previously, was rejected under 35 U.S.C. § 102(b) as being anticipated by each of Duell *et al.* (1992) and Duell *et al.* (1996). Claim 83 has been amended to require a microsomal preparation that includes a recombinant protein expressed by a cell that has been transfected with a nucleic acid molecule encoding the protein, or expressed by a descendent of such a cell. Further the claim requires that the microsomal preparation be substantially free of other proteins that are cytochromes expressed by epidermal cells. See, for example, Figure 13(a) of the application as filed.

None of the Duell *et al.* references teaches or suggests a nucleic acid or protein sequence and so cannot be used to obtain a preparation containing the recited recombinant protein. All of the Duell *et al.* references describe expression of a protein in epidermal (skin) cells only and the expression must be induced by exposure of those cells to retinoic acid. This is much different from Applicants'

invention as claimed in this application, which, through the use of transfection, provides expression of a specific protein without the presence of other cytochromes normally present in skin cells. Moreover, induction of biological activity of this protein by retinoic acid is not required in the transfected cells. An advantage of Applicants' invention as claimed is, for example, in the area of screening drugs (page 9, line 26 to line 40 of the application as filed), where certainty of the identity and presence of the protein being targetted is reproducibly provided, absent contaminating proteins and activities normally present in skin cells. None of the preparations of Duell *et al.* can provide this certainty.

Claim 90, rejected along the same lines as claim 83, has been amended to be directed to a microsomal preparation comprising a recombinant protein which hydroxylates all-*trans* retinoic acid at the C4-position and also requires that the preparation be substantially free of other proteins that are cytochromes expressed by epidermal cells. For the reasons that Applicants believe that claim 83 is patentable over the Duell *et al.* articles and other art of record, Applicants believe that claim 90 is patentable.

Each of independent claims 142 and 150, new in the application, has been drafted to meet the requirements set out in the most recent action. Claim 142 requires the recited protein to oxidize all-*trans* retinoic acid at the C4-position of the ß-ionone ring. Claim 150 requires the recited protein to hydroxylate all-*trans* retinoic acid at the C4-position of the ß-ionone ring. Each claim also requires the recited protein to have a minimum amino acid sequence homology of 60 percent with respect to SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:32, and that the microsomal preparation be substantially free of other proteins that are cytochromes expressed by epidermal cells. Support for these claims can be found, for example, in the third paragraph on page 4 of the application as filed.

The remaining claims, all of which are dependent claims, are being submitted for consideration by the Examiner. These claims recite features described in the application as filed, particularly in the third paragraph on page 4, and at line 6 of page 31. Example 1, on page 17 of the specification, describes BLAST search analyses which revealed less than 30 percent sequence homology between SEQ ID NO:2 (zP450RAI) and other previously known cytochrome P450s. Support for claims 158 to 161 is provided by Examples 6 and 7, and particularly Figures 11(a), 11(b) and 13(a).

Applicants note, as discussed in prior submissions, that the activity of the recited class of protein, and accordingly the claimed microsomal preparation, is not limited to that activity recited in the claims. Referring to Example 3, page 15, lines 10 to 11 of the application, "zP450 expression in COS-1 cells promoted the rapid conversion of RA into both lipid- and aqueous-soluble metabolites." This is evident in Figures 4(a) and 4(b), which are elution profiles of radioactively labelled, lipid-soluble RA metabolites of control and zP450RAI-transfected cells. The profiles clearly demonstrate that zP450RAI produces a significant amount (relative to control) of aqueous soluble metabolites other than (more polar than) RA, 4-OH-RA and 4-oxo-RA. Applicants discuss this in Example 3 (page 15, lines 20 to 23), as follows: "It is possible that the aqueous-soluble radioactivity represents glucuronides of RA metabolites or glucuronides of RA itself. Rapid glucuronidation of 4- and 18-hydroxy-RA in mammalian cell extracts has been reported by others [Wouters, 1992; Takatsuka, 1996]." Applicants thus reasonably expect that a member of the recited class of protein oxidizes the 18-position of the ß-ionone ring as well as the 4-position, and that a said member oxidizes retinol as well as RA (see page 3, lines 23 to 26). In Example 5, Applicants describe on page 17 trials in which the metabolism of radioactively labelled RA by hP450RAI-transfected COS cells was examined.

Results are shown in Figures 10(a), 10(b) and 10(c). All three panels of the figure show radioactive RA-derived metabolites not in the control. hP450RAI produced significant amounts of both aqueous and lipid soluble metabolites. Moreover, at least two unidentified metabolites (i.e., not 4-OH-RA or 4-oxo-RA) were produced. Applicants reasonably believe these to be other retinoid oxidation products. The protein recited in claim 83, for example, thus encompasses a protein that not only oxidizes the C4-position of retinoic acid, but also has other retinoid metabolizing activity.

Applicants make these amendments solely to advance prosecution of this application, and reserve the right to file a continuation or other application as appropriate in order to address otherwise outstanding issues.

Applicants believe that all of the issues addressed in the outstanding Action have been addressed in this response, and thus request allowance of the application.

Note Regarding Representation

The undersigned, an appointed agent of the Applicants, has recently changed firms. The new address and telephone number of the undersigned are indicated below. A change of address of the undersigned is on record with the PTO. A Power of Attorney appointing practitioners under the same PTO Customer Number as the undersigned will be submitted in due course.

In the event that any issue remains, or if the Examiner is disposed to issue an unfavorable final action, the Examiner is invited to telephone the undersigned at (416) 865-8281.

A petition for extension of time for submitting this response, and an authorized Visa Credit Card, Form PTO 2038 accompany this response. Applicants hereby request any further extension of time that may be necessary. Please charge any additional fees which may be required for the papers being filed with this letter to our authorized Visa Credit Card. In the event that charges cannot be made to the authorized credit card, please charge any fee to Deposit Account No. 502651.

Yours very truly,

August 11, 2003
Date

John C. Hunt
Registration No. 36,424

Torys LLP
Suite 3000
79 Wellington Street West
Box 270, TD Centre
Toronto, Ontario
M5L 1A9
Canada

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	Petkovich *et al.*
Serial No.:	09/668,482
Filing Date:	September 25, 2000
Title:	Retinoid Metabolizing Protein
Art Unit:	1652
Examiner:	Elizabeth Slobodyansky, Ph.D.
Atty's Docket No.:	32391-2005

The Commissioner of Patents and Trademarks
Washington, D.C. 20331
U.S.A.

Dear Sir:

This is a submission under 37 C.F.R. § 1.56.

Applicants would like to bring to the attention of the Examiner the enclosed reference entitled, "Retinoic Acid Isomers Applied to Human Skin *in Vivo* Each Induce a 4-Hydroxylase That Inactivates Only *Trans* Retinoic Acid," by Duell *et al.* published in the February 1996 issue of The Journal of Investigative Dermatology.

The Examiner's attention is drawn, in particular, to Figure 1 which shows the time course for induction of RA-4 hydroxylase activity after topical application of 0.1% *t*-RA cream to adult human skin under occlusion.

Yours very truly,

August 11, 2003
Date

John C. Hunt
Registration No. 36,424

Torys LLP
Suite 3000
79 Wellington Street West
Box 270, TD Centre
Toronto, Ontario
M5L 1A9
Canada

PTO/SB/08B (02-03)
Approved for use through 04/30/2003. OMB 0651-0031
U.S. Patent and Trademark Office; U.S.DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	09/668,482
Filing Date	09/25/2000
First Named Inventor	PETKOVICH, Martin P.
Art Unit	1652
Examiner Name	SLOBODYANSKY, Elizabeth
Attorney Docket Number	32391-2005

OTHER PRIOR ART–NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
		ELIZABETH A. DUELL, SEWON KANG, and JOHN J. VOORHEES, Retinoic Acid Isomers Applied to Human Skin in Vivo Each Induce a 4-Hydroxylase That Inactivates Only Trans Retinoic Acid (The Journal of Investigative Dermatology - pgs. 316-320 - Vol. 106, No. 2	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

1 Applicant's unique citation designation number (optional). 2 Applicant is to place a check mark here if English language Translation is attached.

Retinoic Acid Isomers Applied to Human Skin *in Vivo* Each Induce a 4-Hydroxylase That Inactivates Only *Trans* Retinoic Acid

Elizabeth A. Duell, Sewon Kang, and John J. Voorhees
Department of Dermatology, University of Michigan Medical School, Ann Arbor, Michigan, U.S.A.

Application of all-*trans* retinoic acid to human skin for 4 d under occlusion produces a marked increase in retinoic acid 4-hydroxylase activity. In this study, the possible induction of other hydroxylases in response to 9-*cis* and 13-*cis* retinoic acid applications to adult human skin *in vivo* was determined. Application of 0.1% all-*trans*, 0.1% 9-*cis*, and 0.1% 13-*cis* retinoic acid to human skin for 2 d resulted in induction of only all-*trans* retinoic acid 4-hydroxylase activity. The 4-hydroxylase activity in microsomes from the treated tissue ranged from 383 ± 46 to 531 ± 59 pg of 4-hydroxy all-*trans* retinoic acid formed/min/mg protein ($n = 6$). These same preparations were unable to use 9-*cis* or 13-*cis* retinoic acid as substrate for the hydroxylation reaction. Extraction of the retinoic acid isomers from epidermis 48 h after application of 0.1% solution of each isomer yielded significant amounts of all-*trans* retinoic acid (36–72%) regardless of the isomer applied. The all-*trans* isomer produced by isomerization of both 9-*cis* and 13-*cis* retinoic acids is the likely inducer of the 4-hydroxylase. All-*trans* retinol and all-*trans* retinal were unable to compete with all-*trans* retinoic acid as substrate for 4-hydroxylase enzyme. The 4-hydroxylase induced in response to pharmacological doses of retinoic acids is specific for the all-*trans* isomer. The inability of 9-*cis* or 13-*cis* retinoic acid to induce their own hydroxylation and inactivation or act as substrate for the 4-hydroxylase in skin may have considerable implications in light of the clinical use of retinoids in the treatment of various diseases including cancers. *Key words: metabolism/isomerization/pharmacology/cytochrome P450. J Invest Dermatol 106:316–320, 1996*

When human [1] or rat skin [2] is treated topically with all-*trans* retinoic acid (t-RA), a marked induction of RA 4-hydroxylase occurs. Because this hydroxylase may limit the activity of t-RA, 9-*cis* RA, and 13-*cis* RA, we investigated the time course for induction of RA hydroxylase, the specificity of the enzyme with the three isomers of RA, retinol (ROL), and retinaldehyde (RAL) as substrates, and possible induction of other hydroxylases in response to the topical application of 9-*cis* RA or 13-*cis* RA.

MATERIALS AND METHODS

Isocitrate, isocitrate dehydrogenase, NADP, 13-*cis* RA, t-RA, 13-*cis* RAL, 9-*cis* RAL, t-RAL, 13-*cis* ROL, and t-ROL were purchased from Sigma Chemical Co. (St. Louis, MO). Tritiated t-RA, and ^{3}H-t-ROL were obtained from DuPont NEN (Boston, MA). 9-*cis* RA, 9-*cis* ROL, ^{3}H-9-*cis* RA, ^{3}H-13-*cis* RA were gifts from Drs. Joseph Grippo, Arthur Levin, P.F. Sorter, and A.A. Liebman of Hoffmann La Roche Co. (Nutley, NJ). RA metabolites, 4-OH t-RA, 4-oxo t-RA, 4-oxo-13-*cis* RA, and 5,6-epoxy RA, were gifts from Drs. Michael Rosenberger and P.F. Sorter of Hoffmann La Roche. All-*trans* RA 0.1% (Retin-A) and cream vehicle were supplied by Ortho Pharmaceutical Corp. (Raritan, NJ). High-performance liquid chromatography (HPLC) grade solvents were used for extraction and chromatographic solvents. Spherisorb ODS-1 columns were obtained from Phase Separations (Norwalk, CT).

Application of Compounds and Biopsy Procedure Solutions of ROL (1.6%), t-RA (0.1%), 13-*cis* RA (0.1%) and 9-*cis* RA (0.1%) were prepared in a vehicle consisting of 95% ethanol and propylene glycol (7:3, vol/vol) containing 0.5 mg butylated hydroxytoluene/ml of solution (EPG). The use of EGP as vehicle was required since only t-RA was available in cream base used in the initial studies [1]. These solutions and vehicle were applied topically to a 3 × 2-inch area on the buttocks. The area is occluded and kept dark for indicated periods of time, usually 2 or 4 d. The 4-d time point gave consistent clinical evaluations and was the time selected in the initial studies. The 2-d time point, however, is adequate for biochemical studies without clinical correlations. The treated areas were infiltrated with 1% lidocaine local anesthesia and keratomed epidermal tissue (0.1-mm depth) was immediately frozen in liquid nitrogen and stored at −70°C until used. All subjects gave informed, written consent. The study was approved by the University of Michigan Medical Center Institutional Review Board.

Extraction of Retinoids Biopsy sites were tape stripped to glistening prior to biopsy to remove any retinoid trapped in dead stratum corneum that was not absorbed into the viable layers of the skin. Procedures were as previously stated [1]. Briefly, frozen keratome biopsies were ground to a powder under liquid nitrogen with a mortar and pestle. The material was quickly transferred to a homogenizer containing the extraction solvents (chloroform and methanol [2:1]) with tracer ^{3}H-t-RA (to estimate recoveries) and was homogenized. After two additional extractions, the organic phase was evaporated, resuspended, filtered, and injected onto the HPLC column. Protein determinations were by the method of Lowry *et al* [3] with bovine serum albumin as a standard.

Manuscript received February 24, 1995; revised September 27, 1995; accepted for publication October 16, 1995.

This work was presented in part at the annual meeting of the Society for Investigative Dermatology, Baltimore, Maryland (April 27–30, 1994).

Reprint requests to: Dr. Elizabeth A. Duell, University of Michigan Medical Center, Department of Dermatology, R 6558 Kresge I, Box 0528, Ann Arbor, MI 48109-0528.

Abbreviations: t-RA, all-*trans* retinoic acid; 9-*cis* RA, 9-*cis* retinoic acid; 13-*cis* RA, 13-*cis* retinoic acid; ROL, retinol; RAL, retinaldehyde; EPG, 95% ethanol: propylene glycol (7:3, vol/vol) with 0.5 mg butylated hydroxytoluene/ml.

0022-202X/96/$10.50

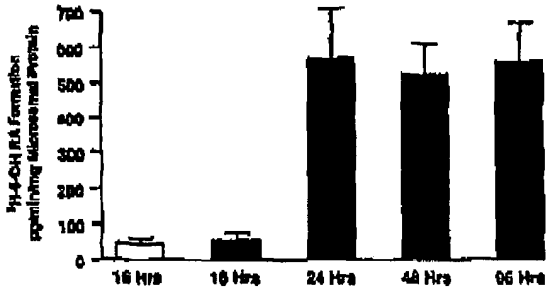


Figure 1. Time course for induction of RA-4 hydroxylase activity after topical application of 0.1% *t*-RA cream to adult human skin under occlusion. ^{3}H-*t*-RA (2 μM) and an NADPH regenerating system was incubated *in vitro* with microsomes from tissues treated *in vivo* with *t*-RA cream. Other assay conditions and separation of metabolites are given in *Materials and Methods*. □, vehicle; ■, samples from *t*-RA sites treated for the times indicated. The number of individual subjects from whom microsomes were prepared at the indicated time points are as follows: n = 6 for vehicle and *t*-RA treated for 16 and 24 h; n = 8 for 48 h; n = 5 for 96 h. *Error bars*, SEM, for each series.

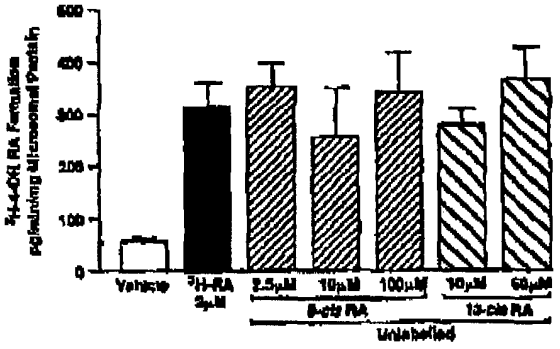


Figure 2. 9-*cis* RA and 13-*cis* RA isomers of *t*-RA are not substrates for RA 4-hydroxylase activity induced by topical application of 0.1% *t*-RA cream. Assay conditions are given in legend to Fig 1 except for the addition of the unlabeled isomers. The number of microsomal fractions used are as follows: vehicle (n = 3), and ^{3}H-*t*-RA (n = 13); for added 9-*cis* RA—2.5 μM (n = 7), 10 μM, (n = 3), and 100 μM (n = 6); for added 13-*cis* RA—10 μM (n = 3) and 60 μM (n = 6). *Error bars*, SEM. Using Student's *t* test for paired samples, there was no significant difference in 4-OH RA formation in the presence or absence of RA isomers ($p > 0.5$).

Enzyme Assays Microsomal fractions were prepared as previously described [1]. RA and RA metabolites were kept in amber glass in darkened rooms with yellow lighting. The rate of RA metabolism was determined by incubating 100 μg microsomal protein in 0.01 M phosphate buffer, pH 7.4, containing an NADPH regenerating system and ^{3}H-*t*-RA or other tritiated retinoids as substrates. Samples were incubated for 30 min at 35°C. The activity is expressed as picograms of 4-OH RA formed per minute per milligram of microsomal protein. Compounds tested as possible competitive substrates were added to the assay mix just prior to the addition of ^{3}H-*t*-RA. The reaction was terminated by addition of 100 μl of methanol cooled to −20°C.

HPLC Separation An HPLC system (1090M; Hewlett-Packard Co., Palo Alto, CA) containing a Spherisorb ODS-1 column (25 × 4.6 mm), a diode array detector and a chem workstation was used to separate and quantitate retinoids and metabolites [1]. The standard compounds used to calibrate the column contained the following retinoids: 4-OH RA; 4-oxo-13-*cis* RA; 4-oxo *t*-RA; 5,6 epoxy RA; 13-*cis* RA; 9-*cis* RA; *t*-RA; 13-*cis* ROL; *t*-ROL; 13-*cis* RAL; *t*-RAL. A gradient elution system separated the retinoids and metabolites in a 42-min time period. Effluent from the HPLC column flowed directly into a flow through scintillation counter (Flo-One Beta model 295A, Packard Instrument Co., Meriden, CT) with a computerized data capture system to determine radioactivity in each peak. The amount of metabolite formed was calculated based on the specific activity of added [^{3}H]retinoid.

RESULTS

Time Course of RA 4-Hydroxylase Induction in Microsomes Isolated from Human Skin Following *In Vivo* Application of 0.1% *t*-RA A marked induction of *t*-RA 4-hydroxylase was reported after human skin had been exposed to 0.1% *t*-RA continuously for 4 d under occlusive patch [1]. Figure 1 shows that in comparison to cream base only (45 ± 15 pg/min/mg microsomal protein), no RA 4-hydroxylase induction occurred at 16 h after application of *t*-RA in cream base (50 ± 18 pg/min/mg protein). In data not shown, there is no significant difference in 4-hydroxylase activity in cream base only treated areas in comparison to areas receiving no treatment. The baseline RA 4-hydroxylase activity varies with different individuals from barely detectable to moderate activity. Twenty-four hours after a single application of *t*-RA in cream base, a 10-fold induction of RA 4-hydroxylase activity (560 ± 142 pg/min/mg protein) had occurred. Similar activities were maintained 48 or 96 h after application of *t*-RA, as shown in Fig 1. The time lag of greater than 16 h but less than 24 h for the increase in RA 4-hydroxylase activity includes the time required for penetration of the *t*-RA, activation of gene transcription, and mRNA and protein synthesis.

9-*cis* RA and 13-*cis* RA as Substrates for RA-4-Hydroxylase in Skin To test whether or not the all-*trans* configuration was necessary for RA to be a substrate for the induced enzyme, nonradioactive 9-*cis* and 13-*cis* RA were added as competitive substrates for the ^{3}H-*t*-RA in the standard *in vitro* assay. The data are given in Fig 2. The ^{3}H-*t*-RA concentration was 2 μM with 4-OH *t*-RA formation of 315 ± 46 pg/min/mg protein. The addition of nonradioactive 2.5 μM 9-*cis* RA, 10 μM 9-*cis* RA, or 100 μM 9-*cis* RA did not significantly decrease the amount of ^{3}H-4-OH *t*-RA formed (average of 317 pg/min/mg protein for all 9-*cis* RA concentrations). Figure 2 also shows that similar results were obtained if nonradioactive 10 μM 13-*cis* RA or 60 μM 13-*cis* RA were present in the assay (average of 323 pg/min/mg protein for both concentrations of 13-*cis* RA). Thus, neither 9-*cis* RA nor 13-*cis* RA were able to compete with *t*-RA as substrate for the enzyme reaction.

In contrast, Fig 3 shows that the addition of unlabeled *t*-RA to 2 μM ^{3}H-*t*-RA decreased the DPM present in the 4-OH *t*-RA and this decrease was proportional to the decrease in specific activity of the ^{3}H-*t*-RA. Based on the specific activity of the substrate, there is no change in enzymatic activity (751 ± 122 pg/min/mg protein

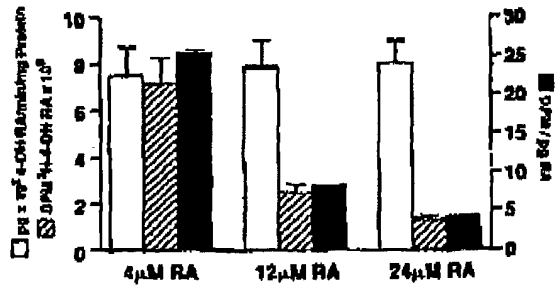


Figure 3. Addition of unlabeled *t*-RA decreases the formation of ^{3}H-4-OH *t*-RA but not the quantity of 4-OH *t*-RA. Assays were as given in legend to Fig 1 except additional unlabeled *t*-RA was added. Formation of 4-OH *t*-RA (pg) was calculated based on specific activity of added substrate. Microsomes were prepared from human skin treated *in vivo* with 0.1% *t*-RA cream ($n = 3$). *Error bars*, SEM.

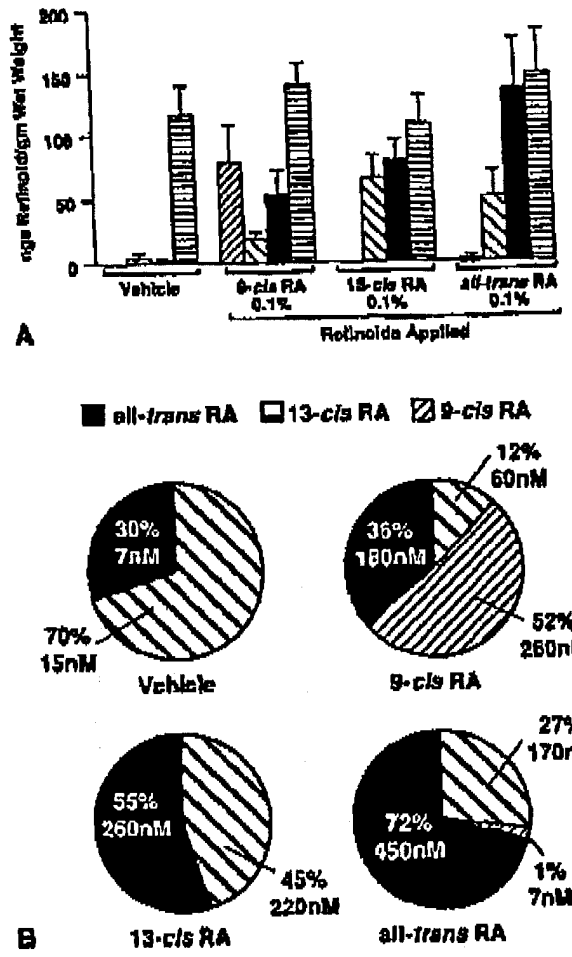


Figure 4. Quantities of 9-*cis* RA, 13-*cis* RA, and *t*-RA extracted from human skin following *in vivo* application in EPG of 0.1% of each isomer for 48 h at different sites on the same individuals. *A*) Nanograms per gram wet weight of each RA isomer and *t*-ROL extracted from the skin. The stratum corneum was stripped prior to keratoming. Extracted material from treated areas: vehicle (□); 9-*cis* RA (▨); 13-*cis* RA (▧); with *t*-RA (■); ROL (▤). *Error bars*, SEM (n = 7). *B*) The percentage of each isomer was calculated based on the sum of nanograms of extracted RAs from each site as 100%. One gram of tissue = 1 ml was used to calculate nanomolars.

at 4 μM RA, 785 ± 112 pg/min/mg protein for 12 μM RA, and 800 ± 96 pg/min/mg protein for 24 μM RA).

Extraction of RA Isomers Present in Epidermis 48 h After *in Vivo* Application of Vehicle, 9-*cis* RA, 13-*cis* RA and *t*-RA to Human Skin All three RA isomers are soluble in EPG at a concentration of 0.1%. Two days after application of vehicle (EPG) and the three RA isomers in EPG to four different sites under occlusion on the buttock, the stratum corneum (outer layers of the skin) was removed by tape stripping. This was necessary in order to avoid erroneously high values for RA content in the viable layers due to unabsorbed material remaining on the surface of the skin.

Retinoic acids were extracted from the stripped, keratomed tissue and the amount of each RA isomer determined. These data are presented in Fig 4*A,B*. The bar graph in Fig 4*A* displays the

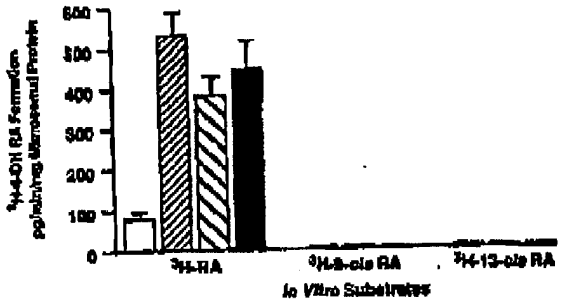


Figure 5. All-*trans* RA 4-OHase activity induced in human skin 48 h after *in vivo* application of 9-*cis* RA, 13-*cis* RA, or *t*-RA. Unlabeled RA isomers were applied *in vivo* in EPG solution for 48 h. All three tritiated isomers were used as substrates in the *in vitro* assay. Assay procedures given in legend to Fig 1 and in *Materials and Methods*. *Error bars*, SEM, for seven different microsomal preparations: vehicle (□); with 9-*cis* RA (▨); with 13-*cis* RA (▧); with *t*-RA (■).

nanograms per gram wet weight of each RA isomer extracted from each of the treated areas. The sum of the nanograms for the three RA isomers for each treated site is defined as 100%. The individual isomers extracted from a given site are expressed as a percentage of the sum and displayed in pie chart form in Fig 4*B*. Molar concentration of each RA isomer is also given for the four treated sites in Fig 4*B*. The total nanograms of the three isomers of RA extracted from the epidermis at each treated site were similar (145–188 ng RAs/g wet weight of tissue). All-*trans* RA (*solid bar*) was the most stable isomer with 72% of the applied isomer remaining as *t*-RA and achieved a concentration within epidermis of 0.45 μM. A significant portion of total retinoic acids extracted from the tissue was *t*-RA regardless of the isomer applied (36% in 9-*cis* RA application areas, 55% in 13-*cis* RA application areas, and 72% in *t*-RA application areas). 9-*cis* RA (*narrow hatched bar*) was recovered from 9-*cis* RA–treated areas (52%) and from *t*-RA–treated areas (1%), but none was detected in the 13-*cis* RA–treated areas. 13-*cis* RA (*wide hatched bar*) was recovered from all of the treated areas.

Induction of 4-Hydroxylase Activity in Epidermis 48 h After *in Vivo* Application of Vehicle, 9-*cis* RA, 13-*cis* RA, and *t*-RA to Human Skin In a second series, the same solutions of the three RA isomers were applied and kept occluded for 2 d. Since RA isomers remaining on the surface would not interfere with either the microsomal preparation or the *in vitro* assay, the stratum corneum was not removed by tape stripping prior to the keratome procedure. The results of the *in vitro* assays for RA 4-hydroxylase activity are depicted in Fig 5. With ^{3}H–*t*-RA as substrate, 4-OH *t*-RA was formed by microsomal fractions from areas treated with each of the three isomers. The activity associated with 9-*cis* RA treated areas was 531 ± 59 pg/min/mg protein, with 13-*cis* RA 349 ± 68 pg/min/mg protein and with *t*-RA 448 ± 68 pg/min/mg protein (n = 7). The vehicle value was 70 ± 16 pg/min/mg protein (n = 7). With ^{3}H–9-*cis* RA or ^{3}H–13-*cis* RA as substrate, no hydroxylated products were formed since no new peaks of radioactivity were detected in the region where more polar compounds elute (i.e., compounds with retention times earlier than 13-*cis* RA [28 min]).

Application of *t*-ROL Increases RA 4-Hydroxylase Activity A solution of 1.6% *t*-ROL in EPG was applied to adult human skin for 4 d under occlusion. Microsomal fractions were prepared from these treated areas and were incubated *in vitro* with ^{3}H–*t*-ROL or ^{3}H–*t*-RA as substrate. As shown in Fig 6, the application of *t*-ROL or *t*-RA induced the *t*-RA 4-hydroxylase activity based on the *in vitro* assay with ^{3}H–*t*-RA as substrate. All-*trans* RA was a much

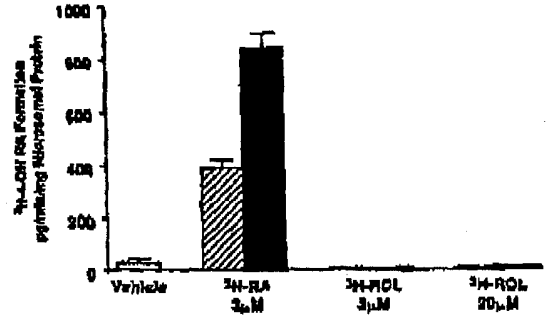


Figure 6. All-*trans* RA 4-OHase activity induced in human skin 96 h after application of 1.6% *t*-ROL or 0.1% *t*-RA. Tritiated *t*-RA or *t*-ROL were substrates in the assay of microsomes obtained from areas treated *in vivo* with *t*-RA or *t*-ROL in EtOH solution. *Error bars*, SEM ($n = 5$, for ^{3}H-*t*-ROL as substrate; $n = 4$, for ^{3}H-*t*-RA as substrate). □, vehicle; ▩, 0.1% *t*-RA; ▨, 1.6% *t*-ROL.

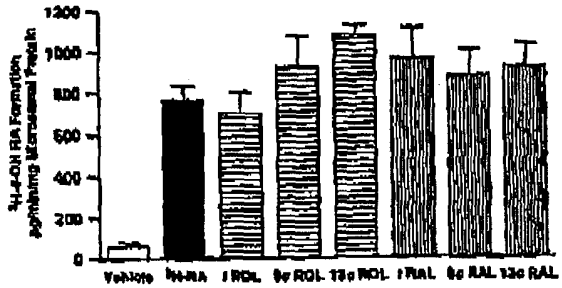


Figure 7. Three isomers of ROL and RAL do not compete with the transformation of ^{3}H-*t*-RA to 4-OH RA *in vitro* with microsomes obtained from areas exposed to 0.1% *t*-RA cream *in vivo* for 96 h. Assays components as given in legend to Fig 1. Unlabeled 9-*cis*, 13-*cis*, and all-*trans* isomers of retinol (20 μM) and retinal (20 μM) were added to the *in vitro* assay in a 7-fold excess of the substrate ^{3}H-*t*-RA. *Error bars*, SEM ($n = 18$, for assays with vehicle or ^{3}H-*t*-RA alone; $n = 6$ for all other experiments given).

more efficient inducer of RA 4-hydroxylase (841 ± 58 pg/min/mg microsomal protein, $n = 4$) in comparison to *t*-ROL (391 ± 32 pg/min/mg protein, $n = 5$). The rate of hydroxylation of *t*-RA in microsomes from vehicle-treated areas was 28 ± 17 pg/min/mg protein ($n = 4$). When microsomal fractions prepared from the ROL-treated areas were assayed for ROL hydroxylase activity, there was no formation of ^{3}H-4-OH ROL since no radioactive peaks were detected at retention times consistent with polar metabolite formation even at a substrate concentration of 20 μM ^{3}H-*t*-ROL. Although 4-OH ROL can be oxidized to additional metabolites in other tissues, no unidentified peaks were present in the chromatograms from these incubations.

Other retinoids tested for their capacity to function as alternative substrate were the other two ROL isomers and the three corresponding RAL isomers. These retinoids (20 μM) were added to the standard *in vitro* assay containing ^{3}H-*t*-RA, which resulted in a 7-fold excess of ROLs or RALs compared with ^{3}H-*t*-RA. The microsomes used in the *in vitro* assays were obtained from areas treated with 0.1% *t*-RA. As shown in Fig 7, there was a 10-fold induction of 4-hydroxylase activity 765 ± 67 pg/min/mg microsomal protein ($n = 18$) for the *t*-RA treated areas compared with 63 ± 14 pg/min/mg protein ($n = 18$) for vehicle-treated areas. There were no significant decreases in the amount of ^{3}H-4-OH *t*-RA formed by the addition to the assay of any retinol or retinal isomer. The activity in the presence of 20 μM *t*-ROL or *t*-RAL was 780 ± 101 pg 4-OH *t*-RA/min/mg protein ($n = 6$) or 938 ± 148 pg/min/mg protein ($n = 6$). These values are not significantly different from *t*-RA alone.

DISCUSSION

The clinical use of retinoids *t*-RA and 13-*cis* RA in topical preparations circumvents one of the cell's control points (the rate of synthesis of RAs) for maintaining the necessary concentration of *t*-RA. Metabolism of the added retinoids to less active metabolites is one of the mechanisms that can be used to limit effects of topically applied retinoids. While little is known about the specifics of retinoid metabolism and inactivation in skin, the induction of a 4-hydroxylase activity in response to topical application of *t*-RA is known [3,2]. A major metabolite of 13-*cis* RA found in blood following oral administration is 4-oxo-13-*cis* RA [4]. a stable 4-hydroxylated metabolite of 13-*cis* RA. *In vitro* incubation of 13-*cis* RA with a 9,000*g* supernatant fraction from rat liver produced the same metabolite [5]. Hydroxylation maybe the main mechanism for inactivation of RAs. The possibility existed that the cytochrome P450 4-hydroxylase induced in response to *t*-RA in skin might also hydroxylate the other isomers (i.e., 9-*cis* RA and 13-*cis* RA) in skin.

The data demonstrate that the 4-hydroxylase induced following application of *t*-RA is specific for *t*-RA. Since retinoids are known to isomerize with exposure to air and light, the stability of each isomer during penetration into the viable layers of the skin was determined. The results show that *t*-RA was the most stable of the three isomers and that regardless of the isomer of RA applied, at least 40–50% of the RA in the viable layers of the epidermis is isomerized to *t*-RA. These data are consistent with recent data obtained with rat liver microsomes *in vitro* [6]. In the report, 40–50% of the added 13-*cis* RA was converted into *t*-RA after a 30-min incubation at 37°C whereas only 11% of added *t*-RA was isomerized into 13-*cis* RA.

The isomerization of the applied 9-*cis* RA and 13-*cis* RA to *t*-RA in the skin would explain the observed induction of the *t*-RA 4-hydroxylase in the epidermis regardless of the isomer applied. A sufficient level of *t*-RA was formed by isomerization of 9-*cis* and 13-*cis* RA so that the same results were produced as if *t*-RA had been applied (i.e., induction of *t*-RA 4-hydroxylase). It is also important to note that neither 9-*cis* RA nor 13-*cis* RA were able to induce a hydroxylase activity in skin that would result in inactivation of these isomers.

Liver, however, does appear able to metabolize 13-*cis* RA by hydroxylation. Experiments with mice showed that feeding 13-*cis* RA increased elimination of 13-*cis* RA only in liver, whereas feeding *t*-RA increased the elimination of *t*-RA in all tissues studied [7]. In other studies microsomal preparations from mouse liver but not mouse skin were able to hydroxylate 13-*cis* RA in an *in vitro* assay system [8].

In human liver cytochrome P450 2C8 hydroxylates not only *t*-RA but also *t*-ROL [9] and rabbit liver P450 2B4 and 1A2 hydroxylate both *t*-ROL and *t*-RAL [10]. Both of these studies were carried out with purified cytochrome P450s. Since topical application of ROL to adult human skin produces many of the same effects as RA [11], we determined whether *t*-ROL could induce a *t*-ROL and/or *t*-RA 4-hydroxylase. Since conversion of ROL to RA may be necessary for induction of RA 4-hydroxylase activity, a much higher concentration of ROL was used to assure adequate penetration and conversion to RA. Topical application of 1.6% ROL, a concentration 16-fold > *t*-RA, did induce RA 4-hydroxylase activity, but to a lesser extent, 46.5% of that achieved with 0.1% *t*-RA. In contrast to the P450s in liver, however, the skin P450 induced by *t*-ROL did not hydroxylate *t*-ROL as indicated by a lack of conversion of ^{3}H-ROL to more polar compounds in the *in vitro* assay system. While 4-OH ROL is less stable than 4-OH RA, the formation of 4-OH ROL with skin as the enzyme source should have been detected if formed since in

vivo assay system was similar to that used to detect 4-OH ROL with liver as the enzyme source [9,10].

The skin appears to tightly control its t-RA levels by strict limitation of its formation from ROL and by specific inactivation through 4-hydroxylation. Skin does not appear, however, to have a direct means of controlling 13-*cis* or 9-*cis* RA levels after topical application, other than isomerization to t-RA or possibly glucuronidation.

Inactivation of applied 13-*cis* RA could be absorption into the blood stream and transport to the liver for conversion to 4-OH-13-*cis* RA by only cytochrome P4502C8 found in liver [9]. Such an inactivation, however, if it occurs in liver, would not be clinically important because topical application of t-RA and 13-*cis* RA in man produces slight and variable changes in blood levels of these retinoids [12,13]. Therefore, local inactivation in the skin of topically applied, naturally occurring stereoisomers of RA via a final common isomerization pathway to t-RA and subsequent 4-hydroxylation appears to be a critical detoxification mechanism in skin. At the present time little is known about the which P450 associated enzyme carries out this reaction in skin. The use of substrates and inhibitor specific for a P450 enzyme family has not shed light on this question (unpublished data). Likewise, the presence of CRABP I or II in the assay did not alter the rate of RA 4-hydroxylation (unpublished results).

Lastly, the kinds of experiments described in this article are difficult to perform in noncutaneous tissues. Nonetheless, should other tissues be similar to skin in their capacity to convert 9- and 13-*cis* RA to t-RA for inactivation, the mechanisms described here may have considerable implications for the treatment of systemic diseases such as aerodigestive tract cancer and acute promyelocytic leukemia with the natural isomers of RA [14,15].

The authors thank Robin Gardener for her expertise in applying the compounds, removing of stratum corneum with tape stripping when required, and keratoming of the treated areas. We are also indebted to Judith Schmitt for her technical expertise and Laura Van Case for the illustrations. Susan Kang acknowledges the support of Dermatology Foundation.

REFERENCES

1. Duell EA, Astrom A, Griffiths CEM, Chambon P, Voorhees JJ: Human skin levels of retinoic acid and cytochrome P-450–derived 4-hydroxyretinoic acid after topical application of retinoic acid *in vivo* compared to concentrations required to stimulate retinoic acid receptor-mediated transcription *in vitro*. *J Clin Invest* 90:1269–1274, 1992
2. Vanden Bossche H, Willemsens G, Janssen PAJ: Cytochrome-P-450–dependent metabolism of retinoic acid in rat skin microsomes: inhibition by ketoconazole. *Skin Pharmacol* 1:178–185, 1988
3. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurements with the Folin phenol reagent. *J Biol Chem* 193:265–275, 1951
4. Boggs CJL, Rodriguez LC, Vane FM: Determination of isotretinoin or etretinate and their major metabolites in human blood by reversed-phase high-performance liquid chromatography. *J Pharm Biomed Anal* 3:269–277, 1985
5. Vane FM, Boggs CJL, Williams TH: Metabolism of 13-*cis*-retinoic acid by a rat liver 9000*g* supernatant preparation. *Drug Metab Dispos* 10:212–219, 1982
6. Sass JO, Forster A, Bock KW, Nau H: Glucuronidation and isomerization of all-*trans*- and 13-*cis*-retinoic acid by liver microsomes of phenobarbital- or 3-methylcholanthrene-treated rats. *Biochem Pharmacol* 47:485–492, 1994
7. Kalin JR, Wells MJ, Hill DL: Effects of phenobarbital, 3-methylcholanthrene, and retinoid pretreatment on disposition of orally administered retinoids in mice. *Drug Metab Dispos* 12:63–67, 1984
8. Oldfield N: The metabolic fate of 13-*cis*-retinoic acid in mouse skin microsomal preparations. *Drug Metab Dispos* 10:1105–1107, 1982
9. Leo MA, Lasker JM, Raucy JL, Kim C-I, Black M, Lieber CS: Metabolism of retinol and retinoic acid by human liver cytochrome P450IIC8. *Arch Biochem Biophys* 269:305–312, 1989
10. Roberts ES, Vaz AD, Coon MJ: Role of isozymes of rabbit microsomal cytochrome P-450 in the metabolism of retinoic acid, retinal and retinol. *Mol Pharmacol* 41:427–433, 1992
11. Kang S, Duell EA, Fisher GJ, Datta SC, Wang ZQ, Reddy AP, Tavakkol A, Yi JY, Griffiths CEM, Elder JT, Voorhees JJ: Application of retinol to human skin *in vivo* induces epidermal hyperplasia and cellular retinoid binding proteins characteristic of retinoic acid but without measurable retinoic acid levels or irritation. *J Invest Dermatol* 105:549–556, 1995
12. Buchan P, Eckhoff C, Caron D, Nau H, Shroot B, Schaefer H: Repeated topical administration of all-*trans*-retinoic acid and plasma levels of retinoic acids in humans. *J Am Acad Dermatol* 30:428–434, 1994
13. Jensen BK, McGinn LA, Kachevsky V, Franz TJ: The negligible systemic availability of retinoids with multiple and excessive topical application of isotretinoin 0.05% gel (Isotrex) in patients with acne vulgaris. *J Am Acad Dermatol* 24:425–428, 1991
14. Lippman SM, Benner SE, Hong WK: Retinoid chemoprevention studies in upper aerodigestive tract and lung carcinogenesis. *Cancer Res* 54:2025s–2028s, 1994
15. Castaigne S, Lefebvre P, Chomienne C, Sue E, Rigal-Huguet F, Gardin C, Dejean A, Archimbaud E, Tilly H, Janvier M, Imbert F, Travade P, Monconduit L, Delannoy A, Rapp MJ, Chebbian D, Montastruc M, Weh H, Fenaux P, Degos L: Effectiveness and pharmacokinetics of low-dose all-*trans* retinoic acid (25 mg/m^2) in acute promyelocytic leukemia. *Blood* 82:3560–3563, 1993

Exhibit C

Page 1 of 3 pages

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/668,482	09/25/2000	P. Martin Petkovich	57600/00035	3039

7590 08/19/2003

John C Hunt Blake Cassels & Graydon
Intellectual Property Group LLP
Commerce Court West
P O Box 25
Toronto, ON M5L 1A9
CANADA

AIR MAIL

EXAMINER	
SLOBODYANSKY, ELIZABETH	
ART UNIT	PAPER NUMBER
1652	14

DATE MAILED: 08/19/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO

EXAMINER	
ART UNIT	PAPER NUMBER

DATE MAILED:

INFORMALITY RE PAYMENT OF FEE

The informality regarding the payment of the fee in connection with ☐ the original filing fee ☐ the amendment filed 8.11.03 is indicated below.

A. FEE DUE

1. ☑ The amendment is considered incomplete in that the funds in Deposit Account No. _____ are insufficient to cover the entire fee due. The balance is due within the period set below.

2. ☐ The amendment is considered an incomplete response, in that payment of $_____ is insufficient to cover the claims as shown in the attached Patent Application Fee Determination Record. Remittance is due within the period set below.

3. ☐ The amendment has not been entered, since applicant has failed to remit (or authorize charge to a Deposit Account) the fee as indicated on the attached Patent Application Fee Determination Record. Remittance or authorization is due within the period set below.

4. ☐ The filing fee of $ 130.00 submitted in this application is insufficient.

   A balance of $_____ is due for additional claims.

5. ☐ a balance of $ 930.00 is due for 3 month 5th time.

APPLICANT IS GIVEN THE REMAINDER OF THE SET PERIOD FOR RESPONSE, OR ONE (1) MONTH FROM THE DATE OF THIS LETTER, WHICHEVER IS LONGER, WITHIN WHICH TO REMIT THE FEE OF $ 1060.00.

B. EXCESS PAYMENT:

5. ☐ It is noted that payment of $_____ is in excess of the amount necessary to cover the claims now in the application. See the attached Patent Application Fee Determination Record.

This matter of refund or credit to your account is being referred to the Finance Officer, for his consid ration.

CLERK OF GROUP

PTOL-319 (REV. 3-82)

USCOMM-DC 32-8838-P82

Exhibit D

Page 1 of 4 pages

CASE No. 32391-2005

SERIAL No. 09/668,482

FILED: 9/25/00

INVENTOR: M. P. Petkovich

The Patent & Trademark Office acknowledges, and has stamped hereon the date of receipt of the items checked below:

[ ] AFFIDAVIT
[ ] AMENDMENT [ ] APPEAL NOTICE
[ ] APPLICATION PAPERS - OATH/DECLARATION
TOTAL CLS. ___ IND. CLS. ___ FEE $___
___ SHEETS OF DRAWING
[ ] ASSIGNMENT [ ] BRIEF
[ ] CERTIFICATE OF CORRECTION
[ ] CLAIM OF PRIORITY AND CERTIFIED COPIES
[ ] DECLARATION
[ ] ISSUE FEE - BASE/BALANCE
[ ] LETTER - CHARGE DEPOSIT ACCOUNT
[ ] LETTER [ ] LICENSE REQUEST
[ ] MOTION
[ ] PETITION
[ ] PRELIMINARY STATEMENT
[ ] INFORMATION DISCLOSURE STATEMENT
[ ] REQUEST FOR EXTENSION OF TIME
[ ] RESPONSE [ ] STIPULATION
[ ] SWORN TRANSLATIONS
[ ] MAINTENANCE FEE TRANSMITTAL
[X] Revocation & Power of
[ ] Attorney, Change of Address

Check No._____ $_____

John C. Hunt

PTO/SB/21 (01-03)
Approved for use through 04/30/2003. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	09/668,482
Filing Date	09/25/2000
First Named Inventor	PETKOVICH, Martin P.
Art Unit	1652
Examiner Name	SLOBODYANSKY, Elizabeth
Attorney Docket Number	32391-2005

Total Number of Pages in This Submission

ENCLOSURES (Check all that apply)

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Response to Missing Parts/ Incomplete Application
  - ☐ Response to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☒ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
- ☐ After Allowance Communication to Group
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to Group (Appeal Notice, Brief, Reply Brief)
- ☐ Proprietary Information
- ☐ Status Letter
- ☐ Other Enclosure(s) (please identify below):

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual	John C. Hunt, Regn. No. 36,424
Signature	
Date	08/20/2003

33721
PATENT TRADEMARK OFFICE

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, Washington, DC 20231 on this date:

Typed or printed			
Signature		Date	

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Commissioner for Patents, Washington, DC 20231.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REVOCATION OF ALL PREVIOUS POWERS OF ATTORNEY AND

NEW POWER OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

The undersigned Queen's University at Kingston, whose full post office address is Kingston, Ontario K7L 3N6, Canada, hereby revokes all previous powers of attorney for the application for a patent entitled **Retinoid Metabolizing Protein**, Serial No. **09/668,482**, which was filed on September 25, 2000, and hereby appoints Conor D. McCourt (Reg. No. 32,385), Eileen McMahon (Reg. No. 33,318), Robin Coster (Reg. No. 38,016), Dolly Kao (Reg. No. 44,451), John C. Hunt (Reg. No. 36,424) and Melanie Sharman Rowand (Reg. No. 48,122) of Tory's LLP, Suite 3000, 79 Wellington Street West, Box 270, Toronto-Dominion Centre, Toronto, Ontario, M5L 1A9, Canada, as its attorneys or agents to prosecute this application, to receive the patent and all correspondence relating to this application, and to transact all business in the United States Patent and Trademark Office connected therewith, with full power of substitution and revocation, and ratifies any act done by the said attorneys or agents in respect of the application.

Please address all correspondence and telephone calls to:

John C. Hunt
Tory's LLP
Suite 3000, 79 Wellington Street West
Box 270, Toronto-Dominion Centre
Toronto, Ontario
M5L 1A9
Canada
Phone 416-865-7500
Facsimile 415-865-7380

Date: 12/08/03

QUEEN'S UNIVERSITY AT KINGSTON

Andrew Simpson
Vice-Principal (Operations & Finance)

Exhibit E

Page 1 of 4 pages

BLAKE, CASSELS & GRAYDON LLP
BARRISTERS & SOLICITORS | PATENT & TRADE-MARK AGENTS

Facsimile: 416.863.2653
Box 25, Commerce Court West
199 Bay Street
Toronto, Ontario, Canada
M5L 1A9

NUMBER OF PAGES INCLUDING COVER PAGE: **DATE:** October 14, 2003

TO:	FACSIMILE:	TELEPHONE:
Dr. John Hunt, Torys LLP, Toronto, ON	416-865-7380	416-865-8121

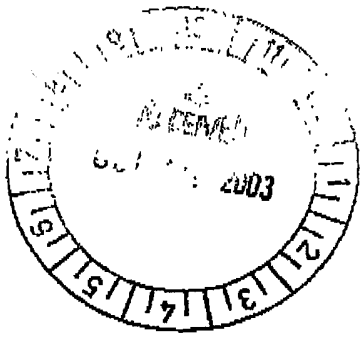
FROM: Sonya Penfold, Ph.D.
BUSINESS PHONE: 416.863.2641
SECRETARY EXT.: 416.863.3182

MESSAGE:

John,

As discussed, here is the Notice received from the USPTO.

Sonya



ORIGINALS SENT BY: (check one) ☐ MAIL ☐ TDX ☐ NOT SENT ☐ _____ (please specify)

ANY PROBLEMS? Please contact your Fax Operator or the Blakes Fax Operator at: 416.863.5842

FILE REFERENCE: ~~47844/1~~ 57600/35

TIME IN: _____ **TIME SENT:** _____ **OPERATOR:** _____

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/668,482	09/25/2000	P. Martin Petkovich	57600/00035	3039

7590 08/19/2003

John C Hunt Blake Cassels & Graydon
Intellectual Property Group LLP
Commerce Court West
P O Box 25
Toronto, ON M5L 1A9
CANADA

AIR MAIL

EXAMINER	
SLOBODYANSKY, ELIZABETH	
ART UNIT	PAPER NUMBER
1652	14

DATE MAILED: 08/19/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address : COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO

EXAMINER	
ART UNIT	PAPER NUMBER

DATE MAILED:

INFORMALITY RE PAYMENT OF FEE

The informality regarding the payment of the fee in connection with ☐ the original filing fee ☐ the amendment filed 8.11.03 is indicated below.

A. FEE DUE

1. ☑ The amendment is considered incomplete in that the funds in Deposit Account No. _____ are insufficient to cover the entire fee due. The balance is due within the period set below.

2. ☐ The amendment is considered an incomplete response, in that payment of $_____ is insufficient to cover the claims as shown in the attached Patent Application Fee Determination Record. Remittance is due within the period set below.

3. ☐ The amendment has not been entered, since applicant has failed to remit (or authorize charge to a Deposit Account) the fee as indicated on the attached Patent Application Fee Determination Record. Remittance or authorization is due within the period set below.

4. ☐ The filing fee of $ 130.00 submitted in this application is insufficient.

   A balance of $_____ is due for additional claims.

5. ☐ a balance of $ 930.00 is due for 3 month S/T extension.

APPLICANT IS GIVEN THE REMAINDER OF THE SET PERIOD FOR RESPONSE, OR ONE (1) MONTH FROM THE DATE OF THIS LETTER, WHICHEVER IS LONGER, WITHIN WHICH TO REMIT THE FEE OF $ 1060.00 .

B. EXCESS PAYMENT:

5. ☐ It is noted that payment of $_____ is in excess of the amount necessary to cover the claims now in the application. See the attached Patent Application Fee Determination Record.

   This matter of refund or credit to your account is being referred to the Finance Officer, for his consideration.

CLERK OF GROUP

PTOL-319 (REV. 9-82)

USCOMM-DC 82-8838-P82

Exhibit F

Page 1 of 2 pages

UNITED STATES PATENT AND TRADEMARK OFFICE

Return To: USPTO Home Page | Finance Home Page

Deposit Account Statement

Requested Statement Month:	August 2003
Deposit Account Number:	502651
Name:	TORYS LLP
Attention:	LISA ANDREW-MCFARLAND
Address:	79 WELLINGTON STREET WEST
City:	TORONTO
State:	
Zip:	M5K 1N2

DATE	SEQ	POSTING REF TXT	ATTORNEY DOCKET NBR	FEE CODE	AMT	BAL
08/25	28	76538495	6100-2087	6001	$335.00	$1,655.00

START BALANCE	SUM OF CHARGES	SUM OF REPLENISH	END BALANCE
$1,990.00	$335.00	$.00	$1,655.00

Need Help? | Return to USPTO Home Page | Return to Office of Finance Home P